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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 17 JUNE 1959 No. 6

CURRENT FOOD TRENDS

The amount, composition and distribution of a nation's food supplies are the basic determinants of the adequacy of its nutritional level.

Suppose we look at the picture for the United States.

Table 1 shows the per capita use of food (retail weight equivalent) by major food groups. The grouping has been done with attention to the kind of nutrients supplied. The table also shows two overall indices which measure changes in estimated pounds of food available, retail weight equivalent, and the per capita food consumption index of the U. S. Department of Agriculture.

Reference to the food consumption index indicates a 10 per cent improvement between 1935–39 and 1947–49, with the index for 1958 at 101 per cent of 1947–49. This index measures food consumption in terms of quality or consumer preference since it adds together the quantities of various foods on the basis of average retail prices paid during the base period, 1947–49.

There was a substantial increase in the consumption of whole milk or its equivalent, eggs, meat and poultry, and citrus between 1935–39 and 1947–49, with a further increase in meat and poultry consumption in 1958. On the other hand, consumption of potatoes and cereal products, including flour, show a continuing decline from 1935–39 into 1958.

Note that in terms of total pounds of food per capita, there was only a 2 per cent increase from 1935–39 to 1947–49, with an estimated decline of 5 per cent from 1947–49 to 1958.

Table 2 translates the average per capita quantities of the various classes of foods shown in table 1 into their nutritional equivalents. As in the case of total pounds, calories show little change from 1935–39 to 1947–49, and a slight decline to 1958. Available protein shows an increase into 1947–49, and this is also true for calcium, iron, thiamine, riboflavin, and niacin. From 1947–49 to 1958, supplies of most nutrients show small change, with the exception of some decline in vitamin A, ascorbic acid and thiamine due in part to the decline in butter and sweet potatoes, the citrus freeze, and the fact that pork supplies were relatively short in 1958.

The last column in table 2 shows the National Research Council's recommended dietary allowances for different age, sex, and occupation groups in 1958, combined into a national per capita average on the basis of population distribution in 1955. These allowances are in terms of *food ingested* and are not directly comparable to the estimated nutrient content of available food supplies. That is, the consumption figures in table 1 have not been discounted for loss of nutrients in the home, whether due to preparation or plate waste. The available supply figures must, of course, exceed the dietary allowances if the Research Council recommendations are to be met.

So far the discussion has been in terms of *overall averages* for the United States. Table 3 summarizes the result of the U. S. Department of Agriculture's 1955 Survey of Household Food Consumption in terms of average nutrient value of diets per person by income class.

A great deal has been written about differences in food expenditures by income classes. Such statements have to do with diet measured in terms of consumer preference and do not necessarily reflect nutritional differences. Table 3 does show, for households of two or more persons by income class,

TABLE 1.—*Approximate consumption of food per capita, retail-weight equivalent, by major food groups, United States*[1]

Item	Unit	1935–39 Average	1947–49 Average	1958 Prel.
Dairy products, excluding butter				
Milk equivalent[2]	Quarts	207	242	247
Product weight	Pounds	376	419	418
Eggs	Pounds	36	47	44
Meat, fish, poultry[3]	Pounds	132	156	169
Fats and oils, including butter, and fat cuts[4]	Pounds	65	65	66
Dry beans and peas, nuts, soya products	Pounds	19	17	17
Potatoes and sweetpotatoes	Pounds	147	123	104
Citrus fruit and tomatoes[5]	Pounds	97	116	109
Leafy, green, and yellow vegetables	Pounds	101	98	81
Other vegetables and fruit	Pounds	238	229	211
Flour and cereal products	Pounds	204	171	148
Sugars and sirups[6]	Pounds	110	109	107
Coffee, tea, and cocoa[7]	Pounds	16	19	17
Total	Pounds	1,541	1,569	1,491
Per capita consumption poundage index	1947–49 = 100	98	100	95
Per capita consumption index[8]	1947–49 = 100	91	100	101

[1] Retail weight represents weight of food as usually purchased in retail grocery stores. Ingredients of bakery products and other mixed food items are shown in their primary food groups.

[2] Computed on basis of calcium content.

[3] Excludes fat cuts of pork; includes edible offals. Meat in terms of fresh retail cuts; fish on edible weight basis; poultry on ready-to-cook basis.

[4] Product weight except for oils used in salad dressing, mayonnaise, etc.

[5] Product weight except for concentrated citrus juices which are on a single strength basis.

[6] To avoid duplication, excludes sugar used in production of canned and frozen fruit, canned fruit juices, canned vegetables, unskimmed, sweetened condensed milk, and frozen dairy products except ice cream.

[7] Includes coffee on roasted basis, chocolate liquor equivalent of cocoa and chocolate products, and tea.

[8] Retail quantities combined using 1947–49 average retail prices.

estimated supplies of various nutrients available from food used in the home during a selected week in April–June 1955. The surprising thing about this set of data is the relatively uniform supply of the several nutrients which are available to families in different income classes. This does not say that nutrition will not be improved by increasing family incomes in many cases, but it does emphasize strongly the fact that much of the malnutrition which exists in the United States today is a matter of food choice or preference rather than an income problem.

Although it is still too early in the year for wholly reliable estimates of food use in 1959, certain significant changes from 1958 are already apparent. Meat consumption is likely to average about 5 per cent higher with practically all the increase in pork. Citrus crops have come through the winter and early spring without major freeze damage, and we should have more normal supplies of oranges than was the case in 1958. Likewise, winter and early spring vegetable crops are also larger than those of a year earlier. The outlook for supplies of other foods, including dairy products, is generally favorable to the continuation of per capita consumption in 1959 at about the same rates as in 1958.

On the basis of these prospective changes, average supplies of several of the important nutrients will also probably show some improvement in 1959. More pork will mean more protein, thiamine, and niacin. More citrus means more ascorbic acid.

In conclusion, some comments may be in order as to probable supplies of poultry and red meat over the next several years. Feed grain supplies are now at a record high and the current prospect is for further increases

TABLE 2.—*Nutrients available for consumption per capita per day and National Research Council recommended dietary allowances, United States*

Nutrient	Unit	Nutrients Available per Capita per Day[1]			Recommended Dietary Allowances per Person, per Day, 1958[3]
		1935–39 average	1947–49 average[2]	1958[3]	
Food energy	Calories	3,310	3,270	3,150	2,400
Protein	Grams	91	95	95	64
Fat	Grams	133	142	144	[4]
Carbohydrate	Grams	446	410	380	[4]
Calcium	Grams	.94	1.04	1.02	.9
Iron	Milligrams	14.5	17.1	16.2	11
Vitamin A value	Int'l. units	8,200	8,200	7,200	4,500
Thiamine	Milligrams	1.47	1.92	1.79	1.2
Riboflavin	Milligrams	1.90	2.34	2.30	1.6
Niacin	Milligrams	16.0	19.6	19.8	17
Folacin	Milligrams	.134	.132	.128	[4]
Ascorbic acid	Milligrams	118	118	106	70

[1] Quantities of nutrients computed by the Institute of Home Economics on the basis of estimates of apparent per capita consumption (retail basis). No deduction has been made in nutrient estimates for loss or waste of food in the home, including preparation and table wastes.

[2] Data for iron, thiamine, riboflavin and niacin include estimates of the quantities of these nutrients added to prepared cereals, white flour, and bread.

[3] Computed by the Institute of Home Economics on basis of National Research Council recommended dietary allowances for 1958 and the population distribution in 1955. *These are in terms of food as ingested.*

[4] Not available.

TABLE 3.—*Average nutritive value by income class of diets per person per day from food used at home in a week, April–June 1955, United States*[1]

Household Size Group and Money Income after Income Taxes	Percent of Population	Food Energy	Protein	Fat	Calcium	Iron	Vitamin A Value	Thiamine[2]	Riboflavin[2]	Niacin[2]	Ascorbic Acid[2]
	Pct.	*Cal.*	*Gm.*	*Gm.*	*Gm.*	*Mg.*	*I.U.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
All households	100.0	3,200	103	155	1.15	17.6	8,540	1.56	2.27	18.7	106
1-person households	2.7	3,470	114	170	1.21	19.9	12,290	1.70	2.49	20.9	143
Households of 2 or more persons	97.3	3,190	103	155	1.15	17.5	8,440	1.56	2.27	18.7	105
Under $2,000	13.7	3,210	93	146	1.11	17.5	7,000	1.58	2.11	17.1	81
$2,000–$2,999	11.4	3,180	100	151	1.11	17.7	8,120	1.58	2.21	18.4	94
$3,000–$3,999	16.9	3,150	100	151	1.12	17.2	8,490	1.54	2.23	18.3	99
$4,000–$4,999	17.3	3,160	104	155	1.17	17.3	8,610	1.56	2.31	18.9	106
$5,000–$5,999	10.6	3,190	107	157	1.18	17.7	8,540	1.56	2.32	19.2	114
$6,000–$7,999	10.7	3,230	109	161	1.18	17.8	8,910	1.56	2.34	19.7	118
$8,000–$9,999	3.6	3,200	107	160	1.16	17.1	8,780	1.49	2.30	18.9	127
$10,000 and over	4.1	3,290	115	169	1.25	18.5	10,340	1.57	2.49	20.4	145
Not classified	9.0	3,180	106	159	1.15	17.6	8,920	1.54	2.30	19.1	116

[1] All data in table refer to housekeeping households which accounted for about 94 per cent of total U. S. civilian population in 1955.

[2] Cooking losses deducted.

in hog numbers in 1959 which would mean another increase in pork for 1960. Meanwhile, marketings of ready-to-cook chicken and turkey, which ran 15.6 pounds in 1935–

39, 22 pounds in 1947–49, and 33.9 pounds in 1958, will apparently show a further increase. Farmers and ranchers increased their numbers of beef cattle and calves by about four million head in 1958, and it appears that a similar increase is in prospect for 1959. This means increased supplies of beef some two, three or four years ahead.

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BURNS AND NEGATIVE BALANCES

There is great interest in improving the recovery of burned patients. Many observations of losses of nitrogen, sodium, potassium and chloride have resulted in attempts to replace these elements during therapy. The fact that the burned animal or patient continues to maintain a negative balance of these elements, frequently in the face of large excesses of added nutrient, is puzzling. This indicates either that there are other unknown essentials being lost that are necessary for retention of the many elements, or that some important metabolic stimulus is lacking.

Some physicians have questioned the wisdom of attempts to overcome these excess losses on the basis that perhaps the process is part of nature's healing technique and should not be interrupted. Nevertheless, efforts are being made continually to reverse the negative balances as they are discovered, with the hope of improving prognosis and accelerating the convalescence of burned patients. Some of the problems have been discussed recently (*Nutrition Reviews* **13,** *202, 295, 345 (1955)*).

B. A. Barnes, O. Cope and R. Moore (*Ann. Surg.* **148,** *755 (1958)*) have reported the results of balance studies of magnesium and other electrolytes carried out in two severely burned patients. Exudates were included in the studies of two other similarly burned patients. Nitrogen balances were also included.

Intake of minerals was determined by using standard food tables listing the composition of foods being eaten. Liquid diets were analyzed directly for magnesium content. Urine specimens were collected daily and analyzed individually. Stool specimens were pooled for periods coinciding with changes in the patients' progress. Exudate losses were derived from analyses of extracts of dressings, the periods depending on the time interval between changes. Since it was not possible to make the stool collection periods coincide with the dressing changes, the balance data showed the effects of daily urinary losses altered by longer periods of stool or exudate losses.

Typical of the usual response to burn injury, the nitrogen losses ranged from 10 g. to 40 g. daily. Conservation of sodium during the first few days in one patient was reversed on the fourth and fifth day when 75 mEq to 350 mEq were excreted as a result of the loss of edema fluid. The second patient showed an unusual retention of sodium, which may have been related to a mild sodium deficiency. The early excretion of potassium was moderate and ranged from 30 mEq to 90 mEq per day. Later losses ranged from 100 mEq to 200 mEq. The daily urinary output of magnesium did not exceed 11 mEq.

The excretion of nitrogen, potassium and sodium in stools was small in comparison to urinary losses. Stool magnesium loss, though variable, was more significant. In Case one the initial loss was about 7 mEq per day. During a moderate diarrhea losses averaged between 30 mEq and 60 mEq per day. In the second patient stool magnesium losses of 50 mEq per day exceeded the magnesium

intake, indicating that losses of the endogenous ion had occurred by way of intestinal secretion.

Exudate losses of nitrogen in Cases one and two averaged less than 5 g. per day. In Case three, however, the daily nitrogen exudate loss was as high as 25 g. per day in some instances. The variation was apparently due to the degree of sepsis, the depth of the burn, or the amount of extracellular fluid and cellular debris.

Sodium to nitrogen losses expressed as mEq per g. showed ratios varying from 0.3 to 3.6. Potassium to nitrogen ratios varied from 0.6 to 2.1. The magnesium to nitrogen ratio was more variable with values ranging between 0.05 and 10.

Several factors influenced the nature and quantity of ingested nitrogen and minerals. During the initial period when plasma and saline were given, the nitrogen and sodium intakes were great. Later the amounts of these elements decreased as the patient was placed on oral feeding. Other factors which caused the intake to vary from day to day were the patients' appetite, food restriction before anaesthesia, and parenteral therapy with plasma, blood or electrolytes.

The ratio of potassium to nitrogen or magnesium to nitrogen excreted served as an indication of the tissue contributing to the catabolism, or benefiting from the anabolism, occurring. Of the 2100 mEq of magnesium in a 70 Kg. adult, 45 per cent is skeletal and only 1 per cent is in the extracellular fluid. The remainder is considered as intracellular. In Case one the magnesium to nitrogen balance ratios ranged between 1 mEq and 16 mEq per gram. Similarly in Case two the negative balance ratio of milliequivalents of magnesium to grams of nitrogen was about six over a 15 week period. The ratio in normal muscle is approximately 0.7. Accordingly, B. A. Barnes, O. Cope and R. Moore were led to propose that skeletal rather than muscular magnesium must be shifting.

The urine losses of magnesium did not influence the balance appreciably but stool losses were more significant.

If one multiplies the volume of gastrointestinal secretions (about 8 L.) by the concentration of magnesium in the extracellular fluid (about 2 mEq per liter) one obtains 16 mEq as the potential magnesium loss. This value is much less than the highest losses occurring and only when isotope tracers studies are used will it be possible to separate endogenous from exogenous magnesium in the stool.

Summary: Balance studies done on two severely burned patients, and analyses of exudates from two other similar patients, revealed that magnesium losses in urine were slight, averaging around 11 mEq per day, and losses in stools were variable but high, averaging as much as 50 mEq per day in some instances. Exudate losses ranged between urinary and stool values, being as much as 40 mEq in some cases. The overall balances of magnesium compared with nitrogen suggested that magnesium shifted in or out of depots other than muscle, possibly to or from bone. Control studies of unburned subjects placed on bed rest should help to indicate the role of inactivity in these shifts of magnesium. Similar shifts of calcium in the bed-ridden patient are well documented and may offer a counterpart of these imbalances in magnesium.

MAPLE SYRUP URINE DISEASE

Many types of inborn errors of metabolism have been discovered and several of these are associated with mental deficiency. Galactosemia, for example, is a disease in which failure of galactose to be converted to glucose causes the accumulation of this sugar resulting in toxic effects (*Nutrition Reviews* **16,** *39 (1958)*).

In Wilson's disease there is a defect in copper metabolism characterized by low serum copper, defective synthesis of serum ceruloplasmin, increased albumin-bound copper, accumulation of copper in liver, brain and other tissues, and an excessive urinary excretion of copper. Other diseases are sucrosuria, familial goitrous cretinism and phenylketonuria.

The study of phenylketonuria has been pursued in great detail in an effort to prevent the mental deterioration common to this disorder. The defect in this metabolic disorder appears to be the failure of conversion of phenylalanine to tyrosine. The direct evidence that this occurs is the accumulation of phenylalanine, the lack of a transient rise in tyrosine after administration of phenylalanine, and a conversion of only small amounts of labeled phenylalanine to tyrosine (not exceeding 10 per cent).

The genetic aspects of metabolic disease in childhood have been summarized recently (*Nutrition Reviews* **16,** *323 (1958)*). The author of this review noted that reduced incidence of serious infectious disease and increased ability to recognize the metabolic diseases have resulted in increasing the number of congenital disease patients until the number is estimated as nearly one third of all patients in a children's hospital at any one time.

J. H. Menkes, P. L. Hurst and J. M. Craig (*Pediatrics* **14,** *462 (1954)*) reported four cases in one family of a severe disorder of the central nervous system, characterized by a maple syrup odor of the urine and early death. D. Y. Mackenzie and L. I. Woolf (*Brit. Med. J.* **1,** *90 (1959)*) have recently described a case of maple syrup urine disease. The patient, a four-month-old girl, stopped eating at one week of age and had to be tube-fed from this time on. At four months she showed jerking movements of the legs and became breathless and cyanotic occasionally. The electroencephalogram showed a severe, generalized abnormality with multifocal discharges typical of metabolic disorders accompanied by seizures. The I. Q. was 55.

Phenylketonuria was excluded by examination of the urine. A heavy precipitate appeared with 2:4-dinitrophenylhydrazine but the ferric chloride test was negative. A maple syrup odor was present. Excesses of leucine, isoleucine and valine were detected in urine, blood, cerebrospinal fluid and saliva using two-dimensional paper chromatography. Keto acids were found in the urine, and non-keto acids with the properties of alpha-hydroxy acids were present. Indoleacetic and indolelactic acids were also present in excess. The blood sugar after a meal was 60 mg. per 100 ml.

D. Y. Mackenzie and L. I. Woolf assumed that this child was suffering from an inborn error in the metabolism of isoleucine, leucine and valine. Amino acids are oxidatively deaminated to form keto acids which may be reduced to form hydroxy acids or combined with coenzyme A prior to decarboxylation. Failure of decarboxylation would cause the accumulation of the amino acids as well as the keto and hydroxy acids. Mackenzie and Woolf indicate that failure to combine with coenzyme A or failure to decarboxylate may have been the specific error in this disease. They suggest that the abnormal metabolites and amino acid excesses could be responsible for the cerebral malfunction. The hypoglycemia was not severe enough to be responsible for the cerebral manifestations.

The authors suggested that a diet low in valine, leucine and isoleucine might be effective in alleviating some of the severe effects of the disorder. Long term administration of such a diet would be economically impractical. Furthermore it is possible that some of the mental deterioration might be irreversible before dietary treatment could be started. Nevertheless, a trial of such a diet was begun in order to determine whether the abnormal amino acids and metabolites would diminish in the urine and serum. The

fact that three essential amino acids are involved in this disease suggests the possibility that the prognosis may be worse than in phenylketonuria, and this is supported by the fact that most cases have died very early.

The diagnosis of maple syrup urine disease is difficult because the disease is easily confused with phenylketonuria. Although acidification with dilute sulfuric acid in the cold enhances the characteristic odor, the possibility of phenylketonuria may be more readily excluded using amino acid chromatography.

This technique was used to analyze the urine and plasma of a case of maple syrup urine disease described by J. Dancis, M. Levitz, S. Miller and R. G. Westall (*Ibid.* **1,** *91 (1959)*). The patient was mentally retarded and died at the age of 20 months. Tissues sampled at necropsy showed transaminase activity indicating that the metabolic block occurred beyond the formation of keto acids. Comparison of a urine specimen collected just before death with a deep-frozen sample collected a year previously showed no elevation of branched chain amino acids in the older specimen, although there was some elevation of keto acids. This indicated that the metabolic block was of long standing and probably represented the primary factor in the disease.

Employing leucine-l-C^{14} made it possible to demonstrate that fresh rat tissue was capable of decarboxylating this amino acid. Tissues from an infant dying of trauma and which were refrigerated in the same manner as those in the case being studied were unable to decarboxylate the labeled amino acid. Therefore further studies of the patient's tissues were abandoned.

Gas phase chromatography demonstrated eight major peaks representing organic acids but the identification was delayed until suitable standards could be acquired. These authors have planned further studies to characterize the hydroxy acids of the branched amino acids in order to locate more exactly the point of metabolic block. One of the problems in the diagnosis of this disease is the fact that a normal amino acid pattern may be found at some stage in the development. A second difficulty is that the maple syrup odor may not always be detected.

Summary: Two cases of patients with maple syrup urine disease have been described in which there was increased excretion in the urine of the branched chain amino acids leucine, isoleucine and valine, as well as their keto acid analogues. The evidence suggests a metabolic block in the normal degradation of the keto acids in these patients.

NEW DIAGNOSTIC TESTS FOR PANCREATIC DISEASE

The diagnosis of chronic disease of the pancreas is often difficult and sometimes impossible. Diagnostic X-ray studies are of value only when calcification or deformation of neighboring organs are visible, usually at an advanced stage of pancreatic disease or tumor. Serum amylase and lipase are transient in their elevations and most of the time are of no help in the diagnosis of chronic disease of the pancreas.

The finding of an elevation of sweat electrolytes in cystic fibrosis of the pancreas has established it as a definite diagnostic procedure for that particular lesion. A simple screening test has even been devised based on the qualitative colorimetric measurement of sweat chloride. An imprint of the palm or foot of the patient to be tested is made on an agar plate in which silver nitrate and potassium chromate have been mixed. The presence of a high chloride content in the sweat is manifested by an intense whitish-yellow discoloration of the imprint on the plate, whereas the normal patient's

imprint is barely perceptible (*New Engl. J. Med.* **255,** *999* (*1956*)).

Such specific diagnostic tests are not available for other pancreatic disturbances. However, recent studies on proteolytic enzymes occurring in the serum and urine appear to afford a new valuable diagnostic aid.

One of these tests (*New Engl. J. Med.* **258,** *797* (*1958*)) involves the titration of serum trypsin using alpha-benzoyl-l-arginine amide hydrochloride as a substrate incubated with the serum to be tested. The ammonia which is formed by hydrolysis of this substrate is determined and is proportional to the amount of trypsin present in the serum. Commercial lyophilized trypsin is used as standard, the maximum normal value being established at 100 units. A study was conducted on 35 control individuals, 16 patients with pancreatitis and seven patients with carcinoma of the pancreas. The average serum trypsin values were, respectively, 31 units, 433 units and 394 units. This test, according to the authors, proved to be a more sensitive index of pancreatic disease than amylase or lipase determination.

Similar results were obtained by other investigators using leucine aminopeptidase determination (*New Engl. J. Med.* **259,** *469* (*1958*)). The substrate used here was L-leucyl-β-naphthylamide hydrochloride, β-naphthylamine being liberated by the enzymatic hydrolysis in amounts proportional to the leucine aminopeptidase activity of the sample tested, urine or serum. The test was applied to normal subjects and to patients with cancer of the pancreas, lymphomas and leukemia, other cancer, pancreatitis, common duct stones, cholecystitis, primary liver disease and to a group of miscellaneous nonmalignant conditions. The leucine aminopeptidase level was significantly increased in both the serum and urine of all of the patients with cancer of the pancreas, whether in the head or in the tail of this organ. Of 110 patients with nonmalignant lesions not involving the gastrointestinal tract, 109 had normal serum levels. Patients with obstruction to the bile flow had elevation of this enzyme level in the serum. Patients with lymphomas and leukemia had increased urinary activity, but little or no increase in serum activity.

Arginine exopeptidase was studied in turn as another possible diagnostic determination for pancreatic disease (*New Engl. J. Med.* **260,** *331* (*1959*)). There the substrate used was benzoyl-l-arginine ethyl ester, the units of the arginine exopeptidase activity representing the number of micromols of the substrate hydrolyzed per hour per milliliter of serum. Again, in this study, it was found that all of the patients who had pancreatitis or pancreatic carcinoma had significant elevations of this enzyme activity in the serum. When compared to serum amylase and lipase determinations, it proved to be a more consistently reliable test.

It appears now that a new set of valuable laboratory tests has become available for the diagnosis of pancreatic disease. It is hoped that this addition will help in the early detection of carcinoma of the pancreas and improve the prognosis of this highly malignant tumor.

THE EFFECT OF NICOTINIC ACID ON SERUM LIPIDS

The fact that it has been reported that nicotinic acid will reduce the level of serum cholesterol (*Nutrition Reviews* **17,** *78* (*1959*)) both in normal individuals and in those having elevated cholesterol levels, led P. A. Gilbraith, W. F. Perry and R. E. Beamish (*Lancet* **I,** *222* (*1958*)) to study the effects of nicotinic acid therapy in normal individuals and those with known atherosclerosis.

These workers determined the serum cholesterol level of both normal subjects (eight

medical students) and 14 patients with atherosclerosis (nine males and five females). The serum cholesterol levels, the total lipids and the β lipoproteins were determined in the serum of both groups of subjects.

Each individual received 3 g. of nicotinic acid per day (1 g. with each meal) while being maintained on his regular diet. It was also noted that none of the subjects had any change in the body weight during the course of the study. The dose of nicotinic acid was in the neighborhood of 200 times that of the suggested daily requirement. In the normal individuals, the serum cholesterol level before giving the nicotinic acid averaged about 180 mg. per 100 ml., and after giving the material for two weeks the cholesterol level had fallen to about 160 mg. per 100 ml. which the authors found significant ($P - 0.05$).

For one week after discontinuing the nicotinic acid, the average cholesterol fell further to a value of approximately 155. From then on, for the next two weeks, the serum cholesterol levels increased until they reached the pre-treatment levels. In the case of the patients with atherosclerosis who received the nicotinic acid for three weeks, there was a marked fall in the serum cholesterol levels from the mean value of about 345 to approximately 255. This, the authors believe to be statistically significant ($P - 0.01$).

It was also noted that there was a reduction in the total serum lipids in both groups. In the normal individuals the β-lipoprotein lipids decreased from 73 per cent of the total lipoprotein lipids to 65 per cent, which the authors believe was significant at the five per cent level. In the case of the patients with atherosclerosis, the β-lipoproteins fraction fell from 80 to 72 per cent of the total lipoprotein lipids at the second week of study. Information beyond that is not given in the short report, but in both cases, upon stopping the nicotinic acid feedings, the lipoprotein levels rose to the pre-treatment level. The mechanism by which nicotinic acid acts in producing decrease in serum lipoproteins or cholesterol levels is not known. But it should be noted that in all subjects this dose of nicotinic acid produced undesirable side effects. There was vasodilatation and pruritis. The effects were less pronounced in the patients with atherosclerosis but the control subjects were very vocal in their complaints. Apparently this is the reason why the control patients were maintained on the nicotinic acid for only two weeks while the patients with atherosclerosis received it for three weeks. These studies agreed with those of others who have shown that nicotinic acid will reduce the cholesterol and lipoprotein fraction of the serum. While nicotinic acid will reduce the serum lipid levels, the administration of nicotinamide has been noted by others to have no effects, and, correspondingly, does not readily induce flushing or pruritis.

The administration of nicotinic acid at this level (3 g. per day) appears to involve some pharmacological property of the compound rather than its vitamin function. It has been noted that the nicotinic acid will produce vasodilatation and pruritis when administered at this level, changes which are not noted when it is administered at vitamin levels.

THE HYPOGLYCEMIC ACTION OF PHENETHYLBIGUANIDE (DBI)

The discovery of the non-sulfonylurea oral hypoglycemic agent, $N_1\beta^1$, phenethylformadinylimourea, known as DBI or Phenformin, was reported by G. Ungar, L. Freedman and S. L. Shapiro (*Proc. Soc. Exp. Biol. Med.* **95,** *190 (1957)*). This agent is a water soluble biguanide which exerts its maximal hypoglycemic action five hours after oral administration. A. N. Wick and C. J. Stewart (*Clin. Res.* **7,** *111 (1959)*),

using C^{14}-labeled DBI, have recovered 90 per cent of the administered dose in the urine in 24 hours, with the tissue concentration localized in the liver and gastrointestinal tract prior to its excretion. It is similar to other biguanides such as Paludrine (N_1-(p-chlorophenyl) - N_5 - isopropylbiguanide) which has been used for many years in the treatment of malaria without any reported hypoglycemic action (L. A. Schmidt, *et al.*, *J. Pharm. Exp. Therap.* **90,** *233* (*1947*)), and is related to the hypoglycemic agent, Synthalin A (R. Bodo, and H. P. Marks, *J. Physiol.* **65,** *83* (*1928*)) which was found 30 years ago to be too toxic to warrant further use.

DBI is effective in lowering blood sugar in most animals including alloxan-diabetic rabbits, rats and monkeys (Ungar, *et al.*, *loc. cit.*), the eviscerated guinea pig (R. L. Nielsen, *et al.*, *Arch. Int. Med.* **101,** *211* (*1958*)) and diabetic man (L. P. Krall, P. White, and R. F. Bradley, *Diabetes* **7,** *468* (*1958*)). S. S. Fajans, *et al.*, (*Clin. Res.* **6,** *252* (*1958*)) have reported extensive studies in normal human subjects which have failed to disclose any evidence of a significant alteration of endocrine function. Glucose tolerance, insulin sensitivity and glucagon - induced hyperglycemia were among the parameters investigated. Death in animals from overdosage seems to be a direct result of hypoglycemia according to Ungar, *et al.*, (*loc. cit.*). High concentrations of the drug also can induce cardiac arrhythmias (A. N. Wick, and E. R. Larson, *Clin. Res.* **6,** *91* (*1958*)). Thus far no deaths and no evidence of renal, hepatic or hematopoetic toxicity attributable to the drug have been reported in man.

The mode, or modes, of action of DBI remain obscure, with lactic acid metabolism the focus of much investigation. Accumulation of lactate in the blood, decrease in muscle glycogen and decrease in muscle oxygen consumption following administration of DBI have led J. M. Tyberghein and R. H. Williams (*Proc. Soc. Exp. Biol. Med.* **96,** *29* (*1957*)) and J. W. Craig, *et al.* (Abstract, 18th meeting *Am. Diab. Assoc.* 6/23/58) to feel that an increase in rate of anaerobic glycolysis may be one action of the drug. Craig, *et al.* (*loc. cit.*) have demonstrated in diabetic patients that fasting blood lactate and total 24-hour urinary lactate are increased following DBI administration. They also found no consistent blood lactate response following glucose infusions but noted increased levels following fructose infusions in diabetic patients receiving the biguanides, compared to control diabetic individuals. These same patients demonstrated a higher and more prolonged blood lactate response to sodium lactate administration than diabetic subjects receiving insulin. Fajans, *et al.* (*loc. cit.*) found increased fasting levels of pyruvate and lactate which were accentuated during glucose tolerance and pyruvate tolerance tests in diabetic patients receiving DBI. These investigators believe that DBI induces a delay in the disappearance of pyruvate and lactate from blood. Their results resemble those recorded by Bodo and Marks (*loc. cit*), who found an increased blood lactate level after Synthalin therapy.

D. F. Steiner and R. H. Williams (*Clin. Res.* **6,** *55* (*1958*)) have suggested that the increase in anaerobic glycolysis after DBI occurs because of a block in the citric acid cycle at the cytochrome level, and A. N. Wick, E. R. Larson and G. F. Serif (*J. Biol. Chem.* **233,** *296* (*1958*)) believe the major block to be in the succinic oxidase system. Nielsen, *et al.*, (*loc. cit*) have shown a decreased release of glucose from the liver of the guinea pig following DBI, but have also demonstrated a hypoglycemic action of biguanide in hepatectomized animals maintained on glucose.

S. S. Bergen, J. G. Hilton and W. S. Norton (*Proc. Soc. Exp. Biol. Med.* **98,** *625* (*1958*)) found no evidence of impairment of adrenal function in diabetic patients responding to DBI therapy. R. H. Williams,

et al. (*Metabolism* **6,** *311* (*1957*)) observed a decrease in liver glycogen and a decrease in urinary nitrogen excretion in guinea pigs given alanine and DBI, indicating a possible inhibition of gluconeogenesis.

Nielsen, *et al.* (*loc. cit.*) reported increased glucose uptake by muscle, and J. Butterfield, *et al.* (*Diabetes* **7,** *449* (*1958*)), using the measurements of peripheral flow and arteriovenous glucose differences, have shown increased peripheral glucose utilization following DBI. R. Tranquada, C. Kleeman and J. Brown (*Clin. Research* **7,** *110* (*1959*)) have studied diabetic subjects by right hepatic vein and femoral artery catheterization over a four-hour period following oral administration of DBI. They found no change in hepatic glucose, urea output, and oxygen consumption but did note a fall in arterial glucose at one and one-half hours. This group interpreted their results as indicating increased peripheral glucose utilization as the cause of the falling blood sugar. In contrast to the observations of Tyberghein and Williams (*loc. cit.*), they were unable to demonstrate changes in blood lactate and pyruvate. These investigators also noted a 30 per cent increase in hepatic blood flow during DBI administration.

Extensive clinical use of Phenformin, including well-controlled studies by T. G. Skillman, *et al.* (*Clin. Research* **6,** *243* (*1959*)) have revealed no serious toxicity thus far. This agent seems to be effective in the milder diabetic patients, with side-effects such as nausea, vomiting, diarrhea and metallic taste often limiting its use more than its failure to be effective. These side-effects quickly abate upon reducing the dose or stopping the drug. Some juvenile diabetic patients have been controlled by DBI alone (Krall, *et al.*, *loc. cit.*), and Skillman, *et al.* (*loc. cit.*) have been unable to administer Phenformin to control the glycosuria of a patient with Cushing's disease. Due to the early occurrence of side-effects of the drug, no human subject has been fully evaluated on biguanide alone following complete pancreatectomy.

In summary, a new oral non-sulfonylurea hypoglycemic agent, DBI or Phenformin, has recently been released for general therapeutic use. While it seems to increase anaerobic glycolysis, the basis of the antidiabetic action remains obscure at present. DBI appears to be useful in the mild diabetic patient and may help stabilize some of the diabetic individuals who are difficult to regulate.

THE CAUSE OF WHITE MUSCLE DISEASE IN LAMBS AND CALVES

White muscle disease of cattle and sheep has eluded practical solution even though it was recognized as a distinct entity more than a quarter of a century ago (H. J. Metzger and W. A. Hagan, *Cornell Vet.* **17,** *35* (*1927*)). Because the degenerative lesions in the muscle are in many ways identical with the lesions that characterize naturally-occurring or experimentally-induced vitamin E-deficiency, some investigators have concluded that white muscle disease represents a vitamin deficiency (K. L. Blaxter, P. S. Watts and W. A. Wood, *Brit. J. Nutrition* **6,** *125* (*1952*); R. Culik, *et al.*, *J. Animal Sci.* **10,** *1006* (*1951*); and W. J. Hartley and D. C. Dodd, *New Zealand Vet. J.* **5,** *61* (*1957*)).

A number of reports have indicated that white muscle disease in lambs or calves could not be considered a simple uncomplicated vitamin E-deficiency (O. H. Muth, *J. Am. Vet. Med. Assn.* **126,** *355* (*1955*); T. B. Keith and A. P. Schneider, *Ibid.* **131,** *519* (*1957*); and J. W. Safford, and K. F. Swingle, *Am. J. Vet. Res.* **16,** *64* (*1955*)). These investigators concluded that the condition appeared to be associated with the

feeding of forage crops, particularly legumes, grown in only certain locations, to cows and ewes during gestation. Blood and feed levels of vitamin E further supported the view that vitamin E-deficiency alone was not responsible for the disease.

A recent report from the Oregon Agricultural Experiment Station, Corvallis, presents evidence to indicate that selenium may be protective against white muscle disease in lambs (O. H. Muth, J. E. Oldfield, J. R. Schubert and L. F. Remmert, *Am. J. Vet. Res.* **20,** *231 (1959)*).

Mature ewes were randomized into five lots of 12 each. Lot one received a ration of one-quarter pound of oats, 2.0 pounds of alfalfa, and 2.0 pounds of Ladino clover, per ewe, per day. The clover was grown in an area where white muscle disease was seldom seen. The basal ration for the remaining four lots consisted of the same level of oats plus 4.0 pounds, per ewe, per day, of a Ladino clover which had been produced under irrigation and in an area where white muscle disease was common. Its alpha-tocopherol content was 25 micrograms per gram and its selenium content was less than 0.1 ppm. The ewes in lot three were injected intramuscularly each week with a water soluble vitamin E (710 I.U. per ewe). The ewes in lot four were each fed 100 I.U. of vitamin E per day as alpha-tocopherol acetate. Those in lot five were fed 0.1 ppm of selenium (as sodium selenite) in the ration.

The ewes were in experiment for 140 days and lambing began on the ninety-fourth day. All lambs were sacrificed at approximately six weeks of age. Tissue sections from the left portion of the heart below the coronary groove were prepared for histological examination as a basis of diagnosis of white muscle disease.

Of 17 lambs born to ewes in lot one (normal hay ration) only one lamb revealed evidence of white muscle disease. In contrast, 11 of 15 lambs in lot two (ration of Ladino clover from an affected area) were affected with the disease. Five of these lambs exhibited clinical signs of stiffness which is characteristic of the more advanced condition. Vitamin E supplementation of the clover ration provided no protection, since 11 of 15, and 16 of 20 lambs in lots three and four, respectively, exhibited signs of the disease. In the lot receiving selenium supplement (lot five) only three of 16 lambs exhibited the disease upon histological examination, and none of these exhibited any gross clinical signs of the disease.

It appears from these results that the minute amount of dietary selenium had a strong protective action against white muscle disease. The authors consider their results as confirming other recent claims of the essential nature of selenium, in minute amounts, as a dietary essential. Selenium has been found to be essential in the diet of rats (K. Schwartz and C. M. Foltz, *J. Am. Chem. Soc.* **79,** *3292 (1957)*); of chicks (E. L. Patterson, L. Milstrey and E. L. R. Stokstad, *Proc. Soc. Exp. Biol. Med.* **95,** *617 (1957)*; K. Schwartz, J. G. Bieri, G. M. Briggs and M. L. Scott, *Ibid.* **95,** *621 (1957)*); and of mice (W. B. DeWitt and K. Schwartz, *Experientia* **14,** *28 (1958)*).

Although, in the past, interest in selenium on the part of animal nutritionists and veterinarians has centered largely on its toxic properties, it is recalled that a growth stimulation in chickens was noted some years ago when the ration was supplemented with sub-toxic levels of selenium (W. E. Poley, W. O. Wilson, A. L. Moxon and J. B. Taylor, *Poultry Sci.* **20,** *171 (1941)*).

It has been known for a number of years that plant uptake of selenium on seleniferous soils can be suppressed by fertilizing the soil with sulphates. Muth and associates have observed that white muscle disease frequently appears following the use of common sulphur-bearing fertilizers. In this connection these authors point out that livestock producers in central Oregon have repeatedly observed that white muscle disease occurred where soil fertilization

with elemental sulphur was practical because of its marked influence in improving crop yields.

Whether or not the conclusions drawn from the results of the Oregon study provide the complete explanation for the cause of white muscle disease in lambs and calves, is perhaps challenged by the observations of workers at the Montana Agricultural Experiment Station, Bozeman, (G. H. Cartan and K. F. Swingle, *Am. J. Vet. Res.* **20,** *235* (*1959*)). These workers reasoned that crops grown on certain soils may be dystrophogenic because of the presence of toxic agents.

On the theory that the initial metabolic defect in the dystrophic muscles may lie in the aerobic phase of muscle metabolism, these workers determined whether substances might be present in dystrophogenic feeds that would interfere with the cytochrome oxidative chain, as measured by the succinoxidase system. An ethanol extract of hay consistently inhibited the succinoxidase *in vitro* system and extracts of hay samples from dystrophogenic areas appeared to exert a greater inhibitory effect than those from hay obtained in areas where the disease was rare. Similar alcohol extracts were also tested for their influence on the xanthine oxidase and cytochrome oxidase systems. The former was unaffected while the latter was inhibited slightly, compared with the inhibition noted in the succinoxidase system.

These observations represent a rather crude attempt to establish the presence of of enzyme-inhibitory substances in dystrophogenic forages. The results, however, suggest that this approach might deserve more critical evaluation. Whether or not the enzyme-inhibitory extracts, when fed, would produce white muscle disease or would influence the succinoxidase system in such animals would be of interest. Whether or not the observed beneficial effect of dietary selenium, as noted by Muth and associates (*loc. cit.*), is in any way related to the observations of the Montana workers also deserves attention.

ARTERIOSCLEROSIS IN ZOO ANIMALS

Arteriosclerosis in mammals and birds at the Philadelphia Zoological Garden has increased 10- to 20-fold during the 40 year period of 1916 to 1956. H. L. Ratcliffe and M. T. I. Cronin (*Circulation* **18,** *41* (*1958*)) have reviewed the autopsy records of a heterogeneous animal population and conclude that dietary factors are not the chief determinants of the increase.

The diagnosis of arteriosclerosis was based on gross and microscopic demonstration of intimal thickening, with or without lipid deposits, and was made in mammals of 45 families (in 360 out of 3,360 autopsies) and in birds of 65 families (in 580 out of 7,660 autopsies). Data are analyzed for successive five year periods.

The authors point out that the introduction of controlled diets in 1935 led to increased longevity, and therefore an increased frequency of lesions. The new diets supplied more fat, but the latter still only comprised 3 per cent of the herbivore diets and 5 per cent of those of omnivores. After the controlled rations were introduced, reproduction and lactation improved; both mammals and birds proliferated and populations became more dense.

Thereafter, reproduction in one group of Capromyidae (which includes the nutria) stopped. Fights became more frequent. Thus social pressures and competition evidently led to a general rise in fatal injuries. Autopsies showed hypertrophy of thyroid and adrenal cortex and suppression of spermatogenesis and oögenesis. Thus, in the last decade the mean age of the autopsied mammals and birds declined, while the

percentage of specimens showing arteriosclerosis increased. Ratcliffe and Cronin therefore suggest that improved diets led to an increase in frequency of arteriosclerosis, either because of "increased life spans of relatively inactive animals, or increased vigor and reproductive drives. The second seems to have been the more important during the last decade of this study, especially during the terminal five-year period".

The authors conclude that "factors other than age or diets may have a major influence" on the development of arteriosclerosis in a wide variety of mammals and birds. They state that lactation failure, suppression of spermatogenesis and oögenesis, and hypertrophy of the adrenal cortex and thyroid glands "are recognized as effects of rapidly developing social pressure".

While the authors found the association of arteriosclerosis and hypertrophy of the zona fasciculata of the adrenal glands only in animals of the Capromyidae, so little is known of the nutritional requirements and pathology of zoo animals that it would be difficult to prove that these findings indeed establish a diagnosis of "social pressure". On the other hand, some groups of inactive animals showed some adrenal cortical atrophy. The complexities of trying to relate adrenal gland morphology to the other factors involved are great, especially in birds.

The authors suggest that the "primary factor in the observed increase in the frequency of arteriosclerosis in this series of mammals and birds has been an imbalance of adrenal secretion". Either inactivity or social pressure might lead to such imbalance. Such a hypothesis is far from proved. However, in preliminary experiments, Ratcliffe and Cronin found arteriosclerosis in confined growing chickens on a low-fat diet, but none in control birds allowed greater space.

While the data recorded in the authors' tables are admittedly heterogeneous and the conclusions are only inferences, the thesis that the increase in arteriosclerosis in zoo animals in the last decade has been independent of diet (and age) appears of interest to nutritional investigators of this disease.

Summary: Arteriosclerosis has increased 10- to 20-fold in frequency in autopsies of Philadelphia Zoo mammals and birds in the period 1916 to 1956. The increase, in the last decade, is apparently independent of the effect of age or diet. "Social pressure", affecting adrenal function, may be the major factor.

REPLACEMENT OF VITAMIN E BY SYNTHETIC ANTIOXIDANTS

It was shown by Dam that certain antioxidants, when added to animal diets deficient in Vitamin E, would delay the onset of deficiency symptoms (*Nutrition Reviews* **10,** *149 (1952)*; **14,** *141 (1956)*). Since then, much effort has been expended in studying the relationship between tocopherol, a naturally occurring antioxidant, and a large number of synthetic antioxidants.

From the practical standpoint, the fact that many synthetic antioxidants will stabilize animal feeds against oxidative deterioration has resulted in the widescale addition of such compounds to commercial feeds. From the biochemical standpoint, the question has been whether these compounds actually substitute for vitamin E, or whether they act by sparing or protecting the vitamin already in the tissues. The latter viewpoint has generally been considered more feasible.

That synthetic antioxidants may have a direct metabolic action of their own has been suggested by a group at the University of Illinois. H. H. Draper, S. Goodyear, K. D. Barbee and B. C. Johnson (*Brit. J. Nutrition* **12,** *89 (1958)*) studied the effectiveness of methylene blue and N,N′-diphenyl-p-phenylenediamine (DPPD) in substituting for vitamin E in the prevention of fetal resorption in rats. Groups of weanling rats

were fed a vitamin E-free purified diet containing 10 per cent of "stripped" lard which was very low in vitamin E. They were divided into six groups of ten rats each.

The weekly oral supplements to three groups were, per rat: 0.7 mg. of DL-α-tocopheryl acetate, 0.7 mg. of D-α-tocopheryl acetate, or 0.4 mg. of DPPD. Two other groups received 0.1 per cent of either DPPD or methylene blue mixed into the diet, and one group received no supplement. After eight weeks they were mated to normal male rats fed a stock diet and then carried through two or more reproductive cycles. The course of pregnancy and the detection of fetal resorption were followed by daily weighings.

In the negative control group of ten rats receiving no supplement, seven became pregnant from the first mating but all resorbed. With the second mating, all ten became pregnant but only seven resorbed. Fifteen pups were born alive. The groups receiving either D- or DL-α-tocopheryl acetate had four and seven pregnancies, respectively, when first mated and no resorptions occurred. At the second mating all 20 became pregnant; only one resorbed. In the group receiving 0.4 mg. of DPPD per week only five of ten rats became pregnant from the first mating, with no resorptions, while the second mating resulted in all of the rats becoming pregnant; there were no resorptions.

The young from the rats fed DPPD were continued on the same diet after weaning, and when eight weeks old were carried through two reproductive cycles. Of five rats bred the first time, four became pregnant and none resorbed. After the second mating, only three were pregnant and none resorbed. A third generation trial on this diet resulted in two of five rats becoming pregnant, but both resorbed.

The groups receiving 0.1 per cent of methylene blue or DPPD in the diet had a very high resorption rate, and will not be discussed in detail.

This experiment indicated that DPPD had a marked effect in preventing vitamin E deficiency in the female rat, but the observation that the protection completely disappeared in the third generation suggests that the antioxidant may have been acting by sparing residual tocopherol, which eventually disappeared.

The experiment, however, is open to two criticisms. In the first place, the investigators did not carry the α-tocopherol group through the same series of matings. Since a purified diet was used, there is the possibility that the failure of reproduction in the later generations may have been due to some dietary deficiency other than vitamin E. In the second place, these workers and others (H. H. Draper, S. Goodyear, K. D. Barbee and B. C. Johnson, *Proc. Soc. Exp. Biol. Med.* **93,** *186 (1956)*; S. R. Ames, M. I. Ludwig, W. J. Swanson and P. L. Harris (*Ibid.* **93,** *39 (1956)*) have shown that DPPD is toxic to the pregnant rat if given at too high a dose, and it is possible that toxicity may have been involved here.

It appeared that if DPPD were completely replacing vitamin E as an antioxidant in the tissues, a better substantiation of this hypothesis would be provided if the DPPD could cure vitamin E deficient rats which had resorbed fetuses one or more times. In this experiment, 4 per cent of cod liver oil was used as the anti-vitamin E stress in place of distilled lard used in the first experiment. A small supplement of methyl linoleate was given, orally after the eighth week when it appeared that the diet may have been low in essential fatty acids. Unfortunately, pregnancies were not recorded in this experiment; only the number of litters delivered and the number of young were noted.

Four groups of 25 rats each, all with no supplement, produced only two litters when first bred at eight weeks of age. During the second reproductive trial, three of the groups received dietary supplements of 0.1 per cent DPPD, 0.01 per cent DPPD, or of

30 mg. D-α-tocopheryl acetate per week. The length of time these supplements were given prior to mating was not stated. The unsupplemented group produced no litters; the DPPD groups produced 15 and 17 litters, respectively, but only 3 and 6 per cent were alive. The vitamin E group produced 19 litters with 71 per cent alive.

During the third reproductive cycle, no supplements were given; the number of litters for the DPPD groups fell to two and one with no live pups. Eight litters came from the vitamin E group with 75 per cent alive. In the fourth cycle, the same supplements were given as in the second cycle. In the group with no supplement, no litters were born. The 0.1 per cent DPPD group (13 rats) delivered five litters with all pups dead. The 0.01 per cent DPPD group (15 rats) produced 8 litters and 32 per cent were born alive. The vitamin E group (18 rats) had 13 litters and 67 per cent were alive.

In a final experiment, another synthetic antioxidant, butylated hydroxytoluene (BHT) was ineffective in substituting for vitamin E when compared with DPPD.

As pointed out above, these studies were complicated by the known toxicity of DPPD for pregnant and new-born rats. Despite this complication, however, it is clear that the role of vitamin E in preventing resorption of fetuses can be taken over by an unnatural antioxidant. It is hardly possible to argue that the DPPD was maintaining sufficient tocopherol in the tissues so that it could still be the active substance, especially since the rats in one experiment were brought out of a condition of almost complete resorption.

In a subsequent paper (H. H. Draper and B. C. Johnson, *Ag. & Food Chem.* **6,** *920 1958*)), these workers have compared the ability of two other synthetic antioxidants related to DPPD in structure to substitute for vitamin E. Although these compounds were not as toxic as DPPD, they were not as effective as DPPD in replacing vitamin E in the rat resorption test. In this study, however, the two new antioxidants, when fed for ten days prior to mating to rats which had previously shown 100 per cent resorption, were able to permit delivery of 12 litters from 75 females. Eighty-seven per cent of the pups were born alive. The unsupplemented group delivered no litters, whereas in the vitamin E-treated group about one-half delivered litters.

Several years ago F. Christensen, H. Dam and R. A. Gortner (*Acta physiol. Scandinav.* **36,** *87 (1956)*) tested methylene blue in the rat resorption test. They tried only one dietary level of the dye and their experimental plan was such that the rats may have had a borderline deficiency of essential fatty acids. Nevertheless, their data indicated that methylene blue had a slight effect in preventing resorption of fetuses.

The studies of Draper, *et al.*, (*loc. cit.*) offer strong evidence for the view that the biochemical function of vitamin E in reproduction may be exclusively that of an antioxidant. Clearly, more studies are needed with a variety of antioxidants at varying doses. It should not be expected that any synthetic antioxidant will be as active physiologically as tocopherol, but the fact that they may replace vitamin E at all would be indicative of an antioxidant mechanism.

EFFECT OF TIME DIETS AND DIET FINENESS ON RAT DENTAL CARIES

The importance of nutrition in the development of sound tooth structure and resistance to dental caries was recognized through the early studies of M. Mellanby (*Brit. Dent. J.* **62,** *241 (1937)*). The impact of maternal diet on caries susceptibility among the offspring has also been recognized. R. F. Sognnaes demonstrated that

weanling rats were more susceptible to caries when the mother had been fed a cariogenic diet during pregnancy and lactation than when the young rats were not subjected to the influence of the cariogenic diet until after weaning (*J. Nutrition* **36,** *1 (1948)*).

The post-weaning age at which cariogenic diets are fed also has been shown to have an influence. Weanling rats exhibited greater caries susceptibility than rats 121 days of age or older (H. C. Hodge, *J. Dent. Res.* **22,** *275 (1943)*; G. E. Braunschneider, H. R. Hunt and C. A. Hoppert, *Ibid.* **27,** *154 (1948)*).

Two interesting reports from the Hawaii Agricultural Experiment Station in Honolulu began with an evaluation of the usefulness of two strains of rats and of the influence of the fineness of the particles in the diet on the initiation and progression of carious lesions under the local laboratory conditions (C. D. Miller and C. A. Schlack, *J. Nutrition* **66,** *105 (1958)*). The basal diet used for these studies had the following composition in parts: Skim milk powder, 350; finely ground whole wheat flour, 650; cornmeal, 150; enriched white flour, 150; brown rice flour, 150; soybean flour, 100; brewers' yeast, 30; cottonseed oil, 46; cod liver oil, 4; salts, 10; and iodized salt, 10. Two strains of rats were used: the one strain had been maintained for more than 25 years in the Hawaiian laboratories and were descendents of the Yale colony; the other strain had been developed at the Naval Medical Institute in Bethesda for caries assays and representatives sent to Honolulu to establish a breeding colony. The mothers of both strains were fed the above diet. At weaning, the litters were divided into two equal groups. One group from each strain was fed the above diet with particle sizes such that all would pass through a 28-mesh sieve. This was called diet 15. The second group was fed the same diet with the particles ground to pass a 48-mesh sieve. The latter diet was designated as 15A.

The Hawaiian strain was very resistant to dental caries when fed both the coarse and fine diets. The Navy strain retained its caries susceptibility when transported to and bred in Hawaii and developed fairly high incidences of carious lesions on both diets. In both strains the coarse diet was more cariogenic. The 28-mesh diet, after 100 days, produced carious scores of 96 and 163 in Hawaiian and Navy strain rats, respectively, contrasted with scores of 36 and 63 for these strains when the 48-mesh diet was fed.

The second study was conducted to learn if feeding a diet with a relatively low caries-producing ability after weaning (21 days old), which is soon after eruption of the first and second molars in rats, would protect the teeth even though they were later subjected to a relatively high cariogenic diet (C. D. Miller, *J. Nutrition*, **66,** *113 (1958)*). The reverse change from a highly cariogenic diet to a diet with comparatively low caries-producing properties as it influenced the condition of the teeth, was also investigated. Diet 15A has already been described in the first paper of the series (Miller and Schlack, *loc. cit.*). In the present paper, this diet is referred to by the author as "non-cariogenic" and "no sugar." In actuality neither of these designations is strictly correct. Wherever the diet was fed, most of the rats in each group developed an appreciable amount of tooth decay. In this regard a more accurate designation for the diet would have been a diet with relatively low caries-producing properties. This diet contained 350 parts of skim milk powder in a total of 1650 parts of all ingredients. Since skim milk powder contains about 50 per cent lactose, the diet actually contained between 10 and 11 per cent of this sugar. Obviously the intention of the "no sugar" designation is that no sugar such as sucrose,

dextrose, fructose, etc. was added to the diet in addition to what is supplied by the natural foods.

The other diet (18A) consisted of the same, finely ground diet except that about 17 per cent by weight of the whole wheat flour was replaced by an equal weight of powdered sugar. Again powdered sugar is loosely used in this manuscript without definition as to whether this is sucrose as available in confectioner's sugar, or sucrose as ground in the laboratory, or some other mono- or disaccharide. The various discussions about the varied cariogenicity of different carbohydrates over the past decade have conditioned any reader in the field of caries research to expect exactness in these matters.

Rats of the Navy strain were used bebecause of their proven susceptibility as demonstrated in the first paper. Eleven litters totaling 77 rats were distributed, by litter, among four treatments as follows: Group one received diet 15A throughout; group two received diet 15A for 42 days after weaning, followed by diet 18A for 48 days; group three received diet 18A for 49 days, followed by diet 15A for 41 days; and group four received diet 18A throughout.

The number of carious molars, the number of carious areas, and the extent of each carious area were recorded and served as criteria for evaluating the cariogenicity of the treatments. The higher the numerical score, the more severe the carious condition.

The influence of added sugar as a cariogenic agent in the diet (diet 18A) when compared with the diet containing only lactose as supplied in skim milk powder (15A) was demonstrated. Rats in group one (no added sugar throughout the entire experimental period) had a caries score of 62, contrasted with a score of 238 when the diet containing added sugar was fed throughout (diet 18A). The results of group two (no added sugar followed by diet with added sugar) and group three (added sugar followed by the diet without added sugar) emphasize that the protective effect of diet is greatest during the immediate post-weaning period. Caries scores were 121 and 199 for groups two and three, respectively. The lower caries score of group three (199) as compared with group four (238) emphasizes that the non-sugar diet was protective even when fed as late as 49 days after weaning.

The experiment was repeated and the post-weaning periods of feeding of both the diet with added sugar and diet without added sugar were extended in the new groups two and three to 56 days; the other diet was then fed for an additional 44 days. A correspondingly greater influence upon the condition of the teeth was demonstrated than had been shown in the first experiment. For example, the caries score was 80 when the diet without added sugar, post-weaning for 56 days, was followed by 44 days of feeding the added sugar diet, contrasted with a score of 344 when the diets were reversed. Actually continuous feeding of the diet with added sugar in this experiment (caries score, 332) was not significantly more damaging than when the added sugar diet was followed by the diet without added sugar (caries score, 344). Since continuous feeding of the diet without added sugar (caries score, 74) yielded essentially the same protection as 56 days of this diet followed by 44 days of the added sugar diet (caries score, 80), the importance of feeding a protective diet during the six or seven week post-weaning period is strikingly illustrated.

Caution must be exercised in the interpretation of these data since the first and second molars were already erupted at the beginning of these experiments. Hence only the third molar could have been under the developmental influence of these diets for as much as two weeks. The remainder

of the influence appears to have occurred through the maturation of the crowns during the early weeks in the oral cavity.

Although rat teeth may be more resistant to a cariogenic diet than are human teeth, experiments such as these help to explain some of the resistance to dental caries shown by sound human teeth when exposed for short or longer periods to caries-producing diets. They also suggest that it is to be expected that non-protective diets during the pre-school period in children, when the permanent teeth are being formed, or possibly more important, in the early months after they erupt, may lead to a high susceptibility to decay in young adulthood. It is evident that relatively good diets in young adults such as were noted among university students in Hawaii, were associated with high dental caries rates, probably because of poor diets during the pre-school period (C. D. Miller, A. C. Bickerton, and G. L. Parke, *Hawaii Med. J.* **11,** *216 (1952)*; R. J. Fanning, *Ibid.* **11,** *214 (1952)*).

The question arises from these human surveys as to whether the diets currently used by these students were good in the nutritional sense as far as their content of required nutrients is concerned, or whether these diets were at the same time deleterious as far as influences upon the oral environment were concerned by reason of high contents of carbohydrates with a low rate of oral clearance. In the production of carious lesions, both caries-susceptible individuals and caries-initiating oral conditions are needed simultaneously. The one without the other normally results in a reduced incidence of dental caries.

PLASMA LIPIDS AND EXPERIMENTAL PANCREATITIS

In the production of experimental pancreatitis in animals, one method is to inject sterile bile into the ligated pancreatic duct. Another is to inject a bland fluid (saline) into the duct. The mechanism of injury is believed to be due to traumatic rupture of the duct-acinar system.

Recently, C. Wang, L. Strauss and D. Adlersberg (*Gastroenterology* **35,** *465 (1959)*) reported that acute pancreatitis could be produced in both rabbits and dogs by injecting a potent staphylococcus toxin into the ligated pancreatic duct. The severity of the pancreatitis could be controlled by varying the volume and concentration of toxin given. Using rabbits, 2.5 ml. of the toxin diluted 1:80 resulted in an acute hemorrhagic pancreatitis with mild general symptoms. All the animals treated in this manner survived. Increasing the concentration of the toxin caused fulminating hemorrhagic pancreatitis and death of the animals.

In their study, 35 rabbits were used. The animals were divided into three groups. The first group consisting of 19 rabbits, had the toxin instilled into the ligated pancreatic duct. Sixteen animals served as controls and this group was further subdivided into three control series. The first control group, consisting of four animals, had the pancreatic duct ligated and received in this duct an amount of saline equivalent to the volume of toxin injected. The second control group (nine animals) had only the pancreatic duct ligated. The third group had the pancreatic duct ligated, and two weeks later received the toxin.

While the production of acute pancreatitis was quite uniform and reproducible in the rabbits, it was much more difficult to establish in dogs using this technique. The variability was believed due to the presence of accessory ducts which led independently into the duodenum. Eleven dogs were studied, of which nine received the staphylococcus toxin and two served as controls and had saline injected into the ligated pancreatic duct.

The lipid concentration in the plasma of these two species was studied. The levels of cholesterol, phospholipids and total lipids were all elevated within one to four days after the instillation of the staphylococcus toxin into the pancreatic duct of both the rabbits and dogs. The maximum increase usually occurred on the second day following the administration of the toxin. An average 4.37-fold increase in the total lipids was found for rabbits by comparing the peak differences between the control group and those receiving the toxin. The average maximum increase in phospholipids and the cholesterol levels was only 2.8-fold. This would indicate that the increase in the plasma triglyceride portion was greater than that in the phospholipid or cholesterol fractions.

By about the eighth to tenth day after injury, the total lipids returned to the control levels and the phospholipid and cholesterol values approached the normal levels much earlier (about the fourth postoperative day). Following instillation of the toxin in the ligated pancreatic duct of dogs, there was also an increase in the plasma lipid fractions.

The maximum increase in plasma total lipids was 1.65-fold, that for phospholipids 1.40-fold, and that for cholesterol 1.81-fold. The authors believe that these differences in response between the dog and the rabbit are due to species differences, although differences in nutritional history were not ruled out. This might be an important variable in the studies involving dogs.

In the two control groups, where the first series consisted of animals which had saline instilled into the ligated pancreatic duct and the second had only pancreatic ligation, both showed some increase in the plasma lipid fraction in comparison to the preoperative period (rabbit: cholesterol +40 per cent, phospholipid +25 per cent and total lipid +29 per cent). There was no significant difference in the lipid values on comparing the effect of duct ligation alone, or ligation plus injection with saline.

A third group of animals had the pancreatic duct ligated and two weeks later the staphylococcus toxin was injected. Severe reactions occurred in this group with one animal dying in 12 hours and the other two sacrificed in moribund condition 24 hours after receiving the toxin. A marked elevation of all the lipid fractions was found, 90 per cent for cholesterol, 50 per cent for phospholipids and 160 per cent for total lipids. Since the animals were in severe shock, the investigators noted that some of these changes might be due to hemoconcentration.

In pancreatitis there is normally an increase in the level of amylase in the serum, though it may only be transient. In all of the animals studied, an increase in the serum amylase was found with much more pronounced increases occurring in the dogs than in the rabbits. A peak of amylase activity was reached on the third day in the rabbits, and then it rapidly diminished. No difference was noted between the animals who received either the toxin or saline by the fourth day. Dogs, on the other hand, had pronounced elevations of serum amylase levels in some cases for as long as 14 days, postoperatively.

In pancreatitis, there is a possibility of destroying islet tissue and this might, in turn, influence the blood sugar levels. The blood sugar was found to be moderately elevated in the rabbits with pancreatitis (an average peak value of 179 mg. per 100 ml) and glycosuria was also noted in three animals. The dog did not show an elevation in blood sugar, and none had glycosuria.

These investigators noted that following an experimentally induced acute pancreatitis in both rabbits and dogs, there is an increase in the plasma lipids. This increase occurs during the period which includes the second to fourth day following the induction of the pancreatitis, then reverts to a normal level. The mechanism of hyperlipemia associated with the pancreatitis is not known. Suggestions have been made that the damage of the alpha cells of the islet tissue may be responsible for the initiation of the hyperlipemia, but as yet this has not been substantiated.

EFFECTS OF CONJUGATED ISOMERS OF ESSENTIAL FATTY ACIDS

There is general agreement that the structure of the essential fatty acids is important for activity in preventing fat deficiency symptoms. Geometric or positional isomers of linoleic, linolenic, or arachidonic acids lack essential fatty acid activity. Whether or not the non-natural isomers are toxic or possess a "stressing" effect on the essential fatty acid deficiency syndrome has not been established with certainty.

When fats or oils are hydrogenated, small amounts of structural isomers of the essential fatty acids are produced. Adverse effects or exaggeration of fat-deficiency symptoms have been noted by some investigators when the diet contained hydrogenated fats, and the isomers of the polyenoic acids were suggested as responsible for such effects (E. Aaes-Jorgensen, J. P. Funch, P. F. Engel and H. Dam, *Brit. J. Nutrition* **10,** *292 (1956);* Funch, Aaes-Jorgensen, and Dam, *Ibid.* **11,** *426 (1957);* and F. Christensen, Dam, and Engel, *Acta physiol. Scandinav.* **38,** *373 (1957)*).

Conjugated linoleic acid, when fed to rats on diets deficient in essential fatty acids, has been noted to hasten the onset of the deficiency (R. T. Holman, *Proc. Soc. Exp. Biol. Med.* **76,** *100 (1951);* Holman and S. I. Greenberg, *Arch. Biochem. Biophys.* **49,** *49 (1954)*). More extensive studies on the effects of conjugated isomers of dienoic and trienoic fatty acids in rats have been reported in a recent paper from the Hormel Institute, and the University of Minnesota (Aaes-Jorgensen, *J. Nutrition* **66,** *465 (1958)*).

Weanling rats were fed a semi-synthetic diet, low in essential fatty acids, with the following percentage composition: vitamin-test casein, 20; sucrose, 70; alpha-cellulose, 4; vitamin and choline supplement in casein, 2; and salt mixture, 4. Five per cent of the sucrose was replaced by an equal amount of ethyl palmitate, cottonseed oil, or hydrogenated coconut oil in three treatment groups comprising six male weanling rats each. In an additional five groups of like number, 5 per cent of sucrose in the diet was replaced by 4 per cent of ethyl palmitate and 1 per cent of one of the following: ethyl linoleate, conjugated *cis, trans*-ethyl linoleate, conjugated *trans, trans*-ethyl linoleate (conjugated dienoic acids), alpha-eleostearate, or beta-eleostearate (conjugated trienoic acids).

After a 17-week experimental feeding period all animals were sacrificed. Heart, testis, brain, and abdominal depot fat samples were analyzed for fatty acids.

During the growth period, examinations were made frequently for essential fatty acid deficiency lesions (scaliness of the skin). A steady increase in skin scaliness occurred in all groups except those receiving ethyl linoleate or cottonseed oil. Judging by the skin score the authors noted rather pronounced evidence of deficiency among the animals receiving the conjugated di- and trienoic acids during the seventh and eighth weeks. However, there was little or no difference in the average skin scores among all the animals not receiving ethyl linoleate or cottonseed oil.

Conjugated dienoic acid concentration in heart lipids was more than doubled in the rats that received the conjugated di- or trienoic acids. Conjugated trienoic acids were not affected by the dietary treatments.

A high concentration of non-conjugated polyunsaturated fatty acids was found in the heart lipids of weanling rats (912 mg. per 100 g. of tissue). The concentration decreased with age in all groups with the greatest decrease (⅓ of initial) occurring in the rats fed the low-fat basal diet. There was no evidence that the conjugated enoic acids in the diet had any influence on the essential fatty acid content of heart lipids.

The non-conjugated dienoic acids in heart lipids were directly influenced by the amount of essential fatty acids in the diet. With trienoic (non-conjugated) acids the reverse was true. That is, when linoleate was fed,

the trienoic acid content was low, whereas when linoleate was not fed, trienoic acid in heart muscle was high. This was a confirmation of earlier observations in the same laboratory (Aaes-Jorgensen and Holman, *J. Nutrition* **65,** *633 (1958);* Aaes-Jorgensen, E. E. Leppik, H. Hayes, and Holman, *Ibid.* **66,** *245 (1958)*).

In contrast to heart lipids, testis lipids were not influenced in their conjugated di- or trienoic fatty acid content by the feeding of such acids. The total polyunsaturates of testis tissue were considerably less (469 mg. per 100 g. of tissue) than those found in heart.

Brain lipids were observed not to accumulate conjugated unsaturated fatty acids. In contrast, adipose (lumbar) tissue from rats fed supplements of conjugated di- or trienoic fatty acids contained large amounts of conjugated dienoic acid, as much as 10 to 12 times the amount seen in animals not receiving the conjugated acid supplement.

Compared with the other tissues analyzed, large amounts of non-conjugated dienoic acids were found in the fat depot of rats fed either palmitate, hydrogenated coconut oil, or the fat-low basal diet. Concentrations of dienoic acid ranged from 159 to 229 mg. per 100 g. of tissue under these conditions. In this connection it should be noted that subcutaneous fat from rats fed fat-free diets has been reported to contain 1 per cent of dienoic acid (H. Dam and P. F. Engel, *Acta physiol. Scandinav.* **42,** *28 (1958)*).

These carefully conducted studies seem to leave little doubt that the pronounced increase in conjugated dienoic fatty acids found in adipose tissue have their origin in dietary conjugated acids. It is of interest that the dienoic acids appeared to be more effective in this regard than the trienoic conjugated acids.

The contrast found between adipose tissue and organ lipids is of interest. The marked increase in non-conjugated trienoic acids in heart and testis lipids, as essential fatty acid deficiency developed, was not seen in adipose tissue. Thus, adipose tissue apparently can not be used for this type of chemical evaluation of fat deficiency.

In the present experiments little, if any, evidence was presented to indicate that the conjugated unsaturated fatty acids had an adverse effect as measured by the skin lesions or the essential fatty acid composition of tissue lipids, even though these acids were fed at a 1 per cent level in the diet. This is many times the amount of conjugated fatty acids that would be consumed in diets containing hydrogenated fats.

BIOTIN DEFICIENCY IN HAMSTERS

Although biotin deficiency has been produced in various laboratory animals by the incorporation of egg white into the diet (M. Sullivan and J. Nicholls, *Arch. Derm. Syph. N. Y.* **45,** *295 (1942)*; J. W. Wilson, E. H. Leduc and D. H. Winston, *J. Nutrition* **38,** *73 (1949)*), data on the biotin requirement of the hamster are inconclusive. B. S. Schweigert (*Vitamins and Hormones* **6,** *55 (1943)*) and J. W. Hamilton and A. G. Hogan (*J. Nutrition* **27,** *213 (1944)*) were unable to establish a definite need. On the other hand, J. M. Cooperman *et al.* (*Proc. Soc. Exp. Biol. Med.* **52,** *250 (1943)*), employing a biotin-free diet, observed retarded growth and dermatitis which were alleviated following administration of 1 microgram of biotin daily.

H. Ranch and W. B. Nutting (*Experientia* **14,** *382 (1958)*) report on the effects of biotin deficiency in the hamster with special reference to the skin and hair. These workers employed two diets: a test diet supplemented with sulfaguanidine and 40 per cent egg white and a control diet lacking these ingredients. Seventy-two hamsters were isolated and weaned at 18 days of age on the test diet, and 33 littermates were maintained on the control diet. In order to establish the

presence of a true biotin deficiency, animals exhibiting extreme symptoms received daily intraperitoneal injections of 4 micrograms of biotin while being maintained on the test diet.

The test diet appeared to cause a rapid depletion of biotin stores so that by six weeks extreme deficiency was apparent. The gain in weight of the vitamin-depleted hamsters was less than half of that of control animals. They were scrawny and exhibited the characteristic kangaroo stance. Other symptoms included nervousness and irritability, jerky movements and a tendency to drag the hind legs. The skin was dry and scaly; the nose and mouth were swollen and the eyes were usually sealed shut with incrustations. Although the animals were almost entirely hairless, tufts or patches of short hairs were sometimes scattered over the dorsum. Individual tufts persisted for a short time, elongating at the normal rate of approximately one millimeter per day, and finally disappearing at about the time full length was attained. The hairs were consistently lighter in color than the normal coat.

Biopsies of the skin showed the epidermis of deficient animals to be noticeably thicker than that of control animals. The thickening was primarily in terms of the stratum corneum although thickening in the stratum germinativum was also apparent. Mitotic figures in the basal layers were plentiful, being slightly more numerous than in normal skin. The epidermis was moderately keratotic and contained many keratohyalin granules. The sebaceous glands were normal for the most part. Hair follicles appeared normal and often exhibited hairs in various stages of development.

Injections of 4 micrograms of biotin per day quickly alleviated the symptoms. During the first week the dermatitis, the swelling of nose and mouth, and the incrustations disappeared. This was accompanied by a sharp increase in weight. By four to six weeks all animals appeared normal, even to the coloring of the hair. In fact, the observation that the color sequence in the hair is so influenced by biotin administration attests to the sensitivity of the process of pigment synthesis.

The skin taken from the biotin deficient animal resembled more those described for the mouse than for the rat. In the hamster the skin was dry rather than covered with brownish incrustations as in the rat. There was only moderate keratosis in the hamster and the mouse as compared to the extensive hyperkeratosis developed in the rat, but the sebaceous glands in the hamster seemed to be less disturbed structurally than in either the mouse or rat.

BIOTIN AND ENZYME ADAPTATION

A. A. Poznanskaia (*Biochemistry* **22,** *668 (1957)*) has observed that amylase synthesis in pancreas slices and serum albumin synthesis in liver slices are both greatly depressed in biotin deficient chicks. It was concluded, however, that biotin did not participate directly in protein synthesis but that its function was concerned with the synthesis of the dicarboxylic acids of the citric acid cycle. These acids were found to be essential for both amino acid synthesis and oxidative phosphorylation. It has also been shown by M. L. Blanchard *et al.* (*J. Biol. Chem.* **187,** *875 (1950)*) that induced adaptive enzyme formation is inhibited in *Neurospora crassa* grown in biotin-deficient media.

In view of these findings, Poznanskaia (*Biochemistry* **23,** *215 (1958)*) has investigated the adaptive formation of the tryptophan oxidase system in livers of biotin-deficient rats. The rats were made biotin-deficient by means of a diet containing dried egg yolk as a source of avidin which forms an inactive complex with biotin. The basic diet

was that described for rats by P. R. MacLeod and H. A. Lardy (*J. Biol. Chem.* **179,** *733 (1949)*). Control animals were fed the same diet but received a subcutaneous injection of 1 μg of biotin daily. Deficient animals developed symptoms of biotin avitaminosis in six to eight weeks, including cessation of growth, weight loss, acute dermatitis and spinal curvature. Tryptophan peroxidase adaptation was studied under the conditions described by E. F. Efimochkina (*Biochemistry* **19,** *68 (1954)*) for tryptophan peroxidase assay. The specific enzymatic activity of the peroxidase was measured as the decrease of added tryptophan during aerobic incubation of aqueous-saline liver extracts.

One hour after the intraperitoneal introduction of DL-tryptophan, the tryptophan peroxidase activity in the livers of biotin-deficient and control animals both increased to the same extent. It follows that biotin deficiency did not affect the adaptive formation of tryptophan peroxidase in the rat. Tryptophan peroxidase activity of liver slices from normal animals increased three- to four-fold on incubation in the presence of tryptophan. Under the same conditions tryptophan peroxidase activity in liver slices from vitamin-deficient rats increased two- to two-and-one-half-fold. Absolute values of tryptophan peroxidase activity in "adapted" liver slices were, however, so low that the difference does not seem significant.

P. R. MacLeod *et al.* (*loc. cit.*) have shown that the rate of ornithine conversion to citrulline is low in liver homogenates from biotin-deficient rats. This phenomenon was used in the present work as a criterion of sufficiently advanced biotin deficiency in the experimental animals. The fraction from normal rat livers synthesized more than twice the amount produced by liver preparations from vitamin-deficient rats.

The author is of the opinion that the adaptive increase in tryptophan peroxidase activity in mammalian livers was not connected with increased protein synthesis *de novo*. His reasons are as follows: (1) N. D. Lee and R. H. Williams (*J. Biol. Chem.* **204,** *477 (1953)*) showed that an increase in tryptophan peroxidase activity was accompanied by a simultaneous loss of protein nitrogen from some cellular structures and an equivalent gain in others. The total protein content of the liver remained unchanged. The authors suggested that the increase in enzymatic activity occurred as a result of some transformation or activation of preformed proteins, or at the expense of the plastic material of structural cellular elements. (2) F. B. Straub (*Minutes Internat. Conf. on Proteins. Libice, Czechoslovakia, 212 (1956)*) found that liver contained a large amount of inactive (masked) tryptophan peroxidase. Under conditions such as paper electrophoresis of liver extract, tryptophan peroxidase activity increased 30- to 40-fold.

Poznanskaia is of the opinion that such unmasking could easily account for the substrate-induced increase in tryptophan peroxidase activity which usually does not exceed seven to ten times the normal value. Poznanskaia also concludes that the inhibition of the adaptive increase of this enzyme in *Neurospora crassa* by biotin-deficiency was a true inhibition of protein synthesis *de novo*, and is therefore distinct from the outwardly similar phenomenon as observed in the mammalian liver.

INADEQUATE PROTEIN INTAKE AND FOOD CONSUMPTION

As nutrition becomes more and more the the study of dietary interrelationships, and less and less the study of simple deficiencies, there is increasing recognition of the need for detailed information concerning factors that affect food intake. Not only is such information essential for the interpretation of many nutrition experiments, but it is also

essential for solving such practical problems as the design of high efficiency diets for commercial animal production.

There have been a number of studies dealing with dietary factors that affect food intake, particularly such factors as the caloric density of the diet and the protein to calorie ratio (*Nutrition Reviews* **12,** *313 (1954)*; **15,** *278 (1957)*). A further study along this line has been reported by J. H. Meyer (*Am. J. Physiol.* **193,** *488 (1958)*). As a result of his observations he has proposed an interesting hypothesis to explain the inability of animals fed on low-protein diets to consume sufficient food to satisfy their protein requirements.

The initial experiments consisted of a study of the effect on food intake, body composition and the dietary level of protein required for maximum growth of adding cellulose to the diet. One series of eight groups of male weanling rats were fed on purified diets containing from 4 to 34 per cent of casein but no cellulose. The other series of eight comparable groups were fed the same diets diluted with 30 per cent of cellulose (70 parts of diet to 30 parts of cellulose) so the protein to calorie ratio remained constant. Food intake, weight gain and carcass moisture content were measured and, using the values for carcass moisture and dry weight, the carcass fat and carcass lean body mass were calculated from an equation relating them to the moisture content of the carcass.

The basic premise in the initial experiments was that, since fiber in the diet causes an increase in the excretion of fecal metabolic nitrogen, the protein requirement should be greater when there is cellulose in the diet. In the series of groups that were fed diets containing no cellulose, 22 per cent of casein was required in the diet to support the maximum gain of 34 g. per week. In the series fed diets containing 30 per cent of cellulose, 26 per cent of casein (expressed as a percentage of the cellulose-free diet) was required to support the maximum gain of 27 g. per week. Over the range from 6 to 22 per cent of casein in the diet, each group fed the basal diet gained more fat-free tissue per gram of casein consumed than did the comparable group fed the same diet diluted with cellulose. This difference is attributed to the greater loss of fecal nitrogen of metabolic origin by animals fed the diets containing cellulose.

On the basis of previous studies by the author (*J. Nutrition* **58,** *407 (1956)*) it was estimated that an additional 1.85 per cent of casein in the diet would replace the metabolic nitrogen lost because of the high intake of cellulose. In the next experiment, therefore, the performance of a group of rats fed a diet containing 14 per cent of casein without cellulose was compared with that of a group fed a diet containing 15.85 per cent of casein and 30 per cent of cellulose. The weight gains of these two groups were similar and their performance was superior to that of a group fed a diet containing 14 per cent of casein and 30 per cent of cellulose.

This was taken as further evidence that the protein requirement is increased by 1.85 per cent owing to the increased loss of metabolic nitrogen as a result of the high cellulose intake. This assumes that the higher *dietary level* of protein required when the diet contains cellulose is indicative of a higher *protein requirement*, an assumption that is not justified. From the data given it can be calculated that the protein *intakes* of these two groups per unit of gain are almost identical and that, if anything, the group consuming the cellulose showed a slightly greater gain per gram of protein consumed.

The same type of experiment was performed using a paired-feeding technique, in which the intake of cellulose-free diet of a group receiving 14 per cent of casein without cellulose and that of a group receiving 15.85 per cent of casein with cellulose were restricted to that of a group receiving 14 per cent of casein with cellulose. The differences in weight gain in this experiment were very small and interpretation is based largely upon differences in fat content of the carcasses calculated from a knowledge of the

moisture content. The 2.0 to 2.5 per cent higher fat content of the carcasses of the rats receiving 14 per cent of casein and cellulose and allowed to eat *ad libitum* is taken as evidence of decreased protein deposition owing to increased loss of metabolic nitrogen. The effect of food restriction as such is not considered. Nevertheless, it is evident from a comparison of the results of the *ad libitum* and pair-feeding experiments that food restriction alone caused a decrease of from 9 per cent to 5 per cent in carcass fat.

In all of these experiments, although food intakes were measured, the interpretation regarding protein utilization is based on protein level in the diet, not upon protein intake. In one instance, as mentioned above, when protein intakes were equal, weight gains and body compositions were similar despite the inclusion of cellulose in one of the diets. The same situation held for groups receiving the lower levels of protein in the first two experiments, *e.g.*, with 10 per cent of casein in the diet no growth-depressing effect of cellulose was observed. This is the protein level at which there was the smallest difference between the food intake of the group receiving no cellulose and that of the comparable group receiving cellulose.

With every other casein level the calorie and the protein intakes of the rats receiving cellulose were below the values for the comparable groups receiving no cellulose. Also, in no case did the growth of any group receiving cellulose equal the value for maximum growth of rats fed on cellulose-free diets, even when the protein intake of those receiving cellulose was well above that required for maximum growth when cellulose was omitted.

These observations suggest that energy, not protein, was the limiting factor for groups receiving cellulose and that the results cannot, therefore, be interpreted as evidence that high bulk causes an increase in protein requirement. It seems probable that the high level of cellulose in the diet prevented the animals from consuming sufficient food to satisfy their energy requirements.

The use of *ad libitum* feeding experiments, in which the protein level of the diet containing cellulose is adjusted upward so that the *protein intakes* of groups having equal weight gains can be compared, seems to be the best approach to the problem. Such comparisons, where they are possible in the present study, suggest that cellulose in the diet has little effect on protein requirement. It would also seem advisable in such experiments to measure carcass composition directly rather than calculate it from the moisture content, particularly when the interpretation of the results may depend upon differences in carcass fat content.

From a study of his own results and those of others, the author proposes an answer to the question of why animals fed low-protein diets do not consume more food to obtain more protein. He notes that the carcasses of animals consuming low-protein diets tend to be higher in fat than those of animals having the same caloric intake but more protein. This suggests that animals on low-protein diets consume more energy relative to their needs and that food intake is inhibited because they cannot dispose of much extra energy.

The higher carcass fat of animals on low-protein diets is taken as evidence of an attempt on the part of the animal to increase its protein intake, a portion of the unnecessary calories consumed along with the protein being deposited as adipose tissue. He cites from a final experiment that cellulose substituted for sucrose stimulates food intake more than cellulose substituted for the entire diet, a result that would be expected when the protein to calorie ratio of the diet is increased so that less calories need be disposed of per unit of protein consumed. Also the rats fed the diet in which cellulose was substituted for sucrose laid down less body fat.

These results could be explained as well,

however, by the hypothesis that food intake is controlled only by caloric need and that when carbohydrate is diluted with cellulose food intake is stimulated because the animal must eat more diet to satisfy its caloric intake and, therefore, in satisfying its caloric intake, ingests more protein.

The main evidence in favor of the first hypothesis is: 1.) that carcass fat deposition was lower when the carbohydrate of the low-protein diet was diluted with cellulose; 2.) that growth was proportional to protein intake rather than calorie intake. These observations support the idea that excess calories are consumed by animals fed low-protein diets. This hypothesis is worthy of further exploration.

This paper illustrates the pitfalls one must be wary of when expressing the protein and amino acid requirements of animals in terms of dietary level. Requirements expressed in this way change when any factor which affects food intake, such as the level of fat or of inert material in the diet, is altered. The hypothesis is presented that the food intake of an animal consuming a low-protein diet may be limited by its capacity to store or dissipate energy.

L-ARGININE AND AMMONIA TOXICITY

The discovery that rats can be protected against the rise in blood ammonia concentration and the toxic reaction resulting from intraperitoneal administration of L-amino acids by the simultaneous administration of L-arginine, suggested that L-arginine should be a component of any amino acid mixture used for intravenous alimentation (*Nutrition Reviews* **15,** *314* (*1957*)). In order to obtain further information about the protective effect of L-arginine J. L. Fahey, R. S. Perry and P. F. McCoy (*Am. J. Physiol.* **192,** *311* (*1958*)) have studied the reactions of dogs to intravenous infusions of mixtures of amino acids.

Female mongrel dogs weighing 9 to 12 kg. were the experimental subjects. They were fed until 18 hours before an experiment and were then allowed only water until the experiment was completed. The dogs were anesthetized and then the solution of amino acids was infused for 60 minutes into a lower extremity vein. The amino acid solution lacking arginine contained, in grams per liter, L-tryptophan, 1.0; L-phenylalanine, 4.4; L-lysine, 3.4; L-threonine, 2.0; L-valine, 3.2; L-methionine, 4.4; L-leucine, 4.4; L-isoleucine, 2.8; and L-alanine, 120.4. Blood samples were taken prior to the start of the infusion and at 20 to 30 minute intervals for four to five hours thereafter. Blood ammonia was determined immediately on one sample and alpha-amino nitrogen and urea were determined on a second sample.

When the amino acid mixture lacking arginine was infused into dogs at a rate of 7.4 to 10.0 mg. of nitrogen per kg. of body weight per minute for one hour, retching and convulsions occurred and death ensued. The infusion of the amino acid mixture without L-arginine at this rate resulted in a steady rise in blood ammonia concentration from between 5 and 10 micrograms of ammonia nitrogen per ml. after one hour to 44 micrograms per ml. three to four hours later, just preceding death. The inclusion of one per cent of L-arginine (molar basis) in the amino acid mixture prevented the symptoms of toxicity and the rise in blood ammonia concentration, as also did the administration of ornithine or citrulline in equivalent quantities. Glutamic acid, however, appeared ineffective in these normal animals, although it has been reported to be of value in cirrhotic patients (*Nutrition Reviews* **15,** *314* (*1957*)).

Infusion of the amino acid mixture lacking arginine at a slower rate (4.6 mg. of nitrogen per kg. of body weight per minute) did not cause the death of the dogs but blood ammonia concentration rose to between 20

and 25 micrograms of ammonia nitrogen per ml. within three hours, then declined to normal values by five hours. Using this procedure it was possible to study the effects of administering arginine at various intervals before and after the amino acid infusion. Administration of L-arginine in an amount equivalent to one per cent, on a molar basis, of the amino acid mixture, prevented the marked rise in blood ammonia concentration. Arginine was effective when given as much as six hours before the infusion, and also was effective in halting and reversing the rise in blood ammonia when administered after the completion of the infusion.

Parallel studies in which ammonium chloride was infused indicated that dogs could tolerate blood concentrations of 20 micrograms of ammonia nitrogen per ml., but that if the concentration exceeded 30 micrograms per ml. convulsions occurred and death ensued unless corrective measures were taken. When the results of experiments on the infusion of L-amino acids were compared with those of experiments on ammonium chloride infusions, evidence was obtained suggesting that as much as half of the amino nitrogen from the amino acids could be released as blood ammonia.

Measurement of urinary urea output and estimation of the total amount of urea present in the body showed very clearly that urea formation was greatly stimulated by the administration of L-arginine. The total urinary ammonia output during the three hours following an infusion was in no case great, generally varying from one to four per cent of the administered nitrogen.

A study of the sequence of formation of ammonia and urea, when no arginine was included in the amino acid mixture and when moderate amounts of the mixture were infused, showed that the alpha-amino nitrogen concentration was maximal at the conclusion of the one-hour infusion. Blood ammonia concentration reached a peak about two hours after the completion of the infusion. Blood urea concentration rose more slowly, the highest values being observed two to three hours after those for ammonia.

These observations, together with the previous studies which show that the capacity of the fasting subject to handle amino acids is restored by the provision of arginine, ornithine or citrulline, emphasize the importance of the Krebs-Henseleit urea cycle as an important metabolic pathway in the mammal. It appears that in the fasting individual the levels of arginine, ornithine and citrulline are so low that large amounts of amino acids cannot be catabolized normally. The fact that a small percentage of arginine corrects this defect is in conformity with the expectation from a knowledge of the urea cycle, namely that arginine should act catalytically. Also, in view of this effect of arginine, it is evident that the enzymes of the system are not seriously depleted during a short period of starvation.

This type of study might well be undertaken using animals on various dietary regimens in order to study the effect of dietary changes on arginine formation and to determine the influence of diet on the susceptibility of the animal to ammonia toxicity.

From a practical point of view, as the authors state, although only eight amino acids may be dietary essentials for man, L-arginine may well be in low enough supply, particularly under conditions of malnutrition or starvation, so that it becomes an essential component for intravenous alimentation.

SORBITOL AND VITAMIN B_{12} ABSORPTION

Observations on the vitamin sparing action of D-sorbitol (Morgan and Yudkin, *Nature* **180,** *543 (1957)*; *Nutrition Reviews* **16,** *126 (1958)*) have stimulated interest in this carbohydrate as a factor influencing intestinal synthesis of vitamins (Jones and Bau-

mann, *J. Nutrition* **66,** *383* (*1958*)) and the absorption of vitamins such as vitamin B_{12} (Greenberg *et al.*, *Nature* **180,** *1401* (*1957*)) in animals. During the past year studies on vitamin B_{12} absorption have been extended to normal human subjects and to subjects suffering from pernicious anemia.

The interest of B. F. Chow and associates in the problem of factors affecting vitamin B_{12} absorption was broadened when they observed that an experimental lipotropic elixir enhanced the absorption of vitamin B_{12} in man and in the rat (*Am. J. Clin. Nutrition* **4,** *434* (*1956*)). They later studied the effects of the individual components of the lipotropic elixir (B. F. Chow, P. Meier and S. M. Free, Jr., *Ibid.* **6,** *30* (*1958*)) on vitamin B_{12} absorption.

Clinically healthy men consuming an adequate diet were used as the subjects in their experiments. Vitamin B_{12} absorption was estimated using the technique of Schilling (*J. Lab. Clin. Med.* **42,** *860* (*1953*)). Each subject was given 10 ml. of a solution containing 50 micrograms of radioactive vitamin B_{12}, and two hours later 1 mg. of unlabeled vitamin B_{12} was injected intramuscularly to flush out the radioactivity. Urine was collected for 24 hours and the radioactivity excreted in the urine was taken as a measure of absorption. The experiments were carefully controlled to minimize the influence of subjective factors and time variations.

In preliminary tests the average urinary vitamin B_{12} excretion was highest when the vitamin was administered with the full lipotropic formula and appeared to be higher when some of the individual vitamins were included than when they were omitted. Subsequent studies with individual vitamins administered with sorbitol suggested that niacinamide influenced vitamin B_{12} absorption. However, when the effect of niacinamide was studied in detail with and without sorbitol it became apparent that niacinamide was without effect when the sorbitol was omitted. In a final experiment in which the effect of sorbitol alone was studied, the urinary excretion of the orally administered vitamin B_{12} was 581 millimicrograms in 24 hours for nine control subjects and 852 for eight subjects receiving D-sorbitol as well. This difference was significant.

In a further study (Chow *et al.*, *Am. J. Clin. Nutrition* **6,** *386* (*1958*)) the serum vitamin B_{12} levels of subjects receiving 25 micrograms of vitamin B_{12} per day administered orally in a capsule containing sorbitol were found to be considerably higher after four months of treatment than those of comparable subjects receiving 100 micrograms of vitamin B_{12} without sorbitol.

Further studies on the effect of sorbitol on vitamin B_{12} absorption in rats have been reported by E. G. Rice, J. F. Herndon, E. J. Van Loon and S. M. Greenberg (*Am. J. Physiol.* **193,** *513* (*1958*)). They used female rats in their study. These were separated into three groups. The first group consisted of a non-pregnant control group and the second group was placed on test seven days after conception, as was the third group 14 days after conception. One half of each group received radioactive (Co^{60}) vitamin B_{12} orally with water; the other half received an equal amount of the radioactive vitamin in a 28 per cent solution of D-sorbitol daily for three days. Forty-eight hours after the final dose, a blood sample was removed from each rat. The animals were then killed; the kidneys, liver, gastrointestinal tract and uterus (with fetus in pregnant animals) were removed, they were solubilized in potassium hydroxide and the radioactivity was counted.

In every case more radioactivity from vitamin B_{12} was found in the blood, kidneys, livers, and gastrointestinal tracts of rats receiving sorbitol, indicating again the enhancing effect of sorbitol on vitamin B_{12} absorption. Pregnancy, in agreement with observations of Hellegers *et al.*, (*Am. J. Clin. Nutrition* **5,** *327* (*1957*)) also increased vitamin B_{12} absorption but not to the extent that D-sorbitol did. The results also show very clearly the shift of vitamin B_{12} from the

maternal tissues to the fetus during the third trimester of pregnancy, but even here the effect of administering D-sorbitol was marked. Fetuses of mothers receiving sorbitol contained about 7 per cent of the administered radioactivity whereas those from mothers receiving no sorbitol contained only about 2.5 per cent of the administered radioactivity. This provides support for the observation of Izak *et al.*, (*Arch. Int. Med.* **99,** *346 (1957)*) that the great fetal demand for vitamin B_{12} may lead to the development of vitamin B_{12} deficiency in the pregnant female.

The question of the mechanism of the effect of D-sorbitol on vitamin B_{12} absorption and its relation to the impaired absorption that is responsible for pernicious anemia has been raised by Schilling (*Am. J. Clin. Nutrition* **6,** *332 (1958)*) and by Barnard (*Ibid.* **6,** *333 (1958)*). A comparison of the effects of D-sorbitol in normal and in pernicious anemia patients, it is pointed out, would provide information about this problem.

Such information has been provided by L. Ellenbogen, V. Herbert and W. L. Williams (*Proc. Soc. Exp. Biol. Med.* **99,** *257 (1958)*), who have studied the effects of D-sorbitol on the absorption of Co^{58} labeled vitamin B_{12}. In two experiments with pernicious anemia patients these workers administered either 2 micrograms or 30 micrograms of vitamin B_{12} orally, either with or without 10 g. of D-sorbitol. The urinary excretion of the radioactivity was measured following administration of one mg. of unlabeled vitamin B_{12}. In no case was there any evidence that sorbitol enhanced vitamin B_{12} absorption in any of these patients, and in some cases there was evidence that D-sorbitol inhibited absorption of the 30 microgram dose. Lower levels of sorbitol were also ineffective.

In a second series of trials the effect of D-sorbitol was studied when similar doses of vitamin B_{12} were administered with intrinsic factor. Intrinsic factor greatly enhanced the absorption of the higher doses. In no instance was the effect of intrinsic factor enhanced by the simultaneous administration of D-sorbitol.

These results indicate, as emphasized by Herbert (*Am. J. Clin. Nutrition* **6,** *547 (1958)*), that D-sorbitol is unlikely to be of value in the treatment of pernicious anemia.

To summarize briefly, it is evident that D-sorbitol enhances the absorption of vitamin B_{12} in rats and in normal human subjects. Evidence has been presented, however, indicating that it is ineffective in pernicious anemia patients.

NOTES

Allergic Reactions to Penicillin-Containing Milk

M. C. Zimmerman (*A. M. A. Arch. Dermal.* **79,** 1 (1959)) has reported four patients with proven allergic reactions to penicillin in ingested dairy products. Since penicillin allergy can be fatal, and reactions are becoming more numerous in the population, it is of interest that as many as 11 per cent of milk samples contain measurable amounts of penicillin (*loc. cit.*). This is used to treat the very common mastitis of cows, by catheter instillation up the teat duct. High dosages have been reduced by regulation of the Food and Drug Administration, but failure to discard milk for three days after treatment has led to economic loss in the cheese industry, for cheese-making bacteria are inhibited. Thus the problem is not academic.

Zimmerman's patients were individuals with known reactions from a previous penicillin injection who later developed hives every time they drank milk or ate dairy

products. Roquefort or other cheeses containing *Penicillium* mould were thought particularly troublesome. Though penicillin in milk has frequently been indicted as a cause of human allergic reactions, the author believes he has in this case proved his diagnosis. Each of his patients had a "typical" penicillin reaction, duplicated repeatedly by eating dairy products, clearing rapidly after an injection of the enzyme penicillinase, and each could eat dairy products without allergic reaction when injected in advance with penicillinase. The case summaries make exceedingly interesting reading.

Efforts by the Food and Drug Administration are continuing to eliminate the abuses; a dye is being sought which, when incorporated with the penicillin, would discolor milk for several days, forcing its discard.

Lack of Increased Clearing Factor Inhibitor in Atherosclerosis

Heparin and some sulfated polysaccharides clear lipemic serum *in vivo* by causing the secretion of a "clearing factor", presumbly a lipoprotein lipase. The phenomenon has been studied in itself (*Nutrition Reviews* **12,** 294 (1954); **14,** 216 (1956)), and in relation to experimental atherosclerosis (*Ibid.* **13,** 20 (1955); **15,** *187* (1957)). Clearing factor is present in normal plasma and tissues; since its elaboration has been found to be reduced in human atherosclerosis, experimental nephrosis, and diabetes, it may be etiologically concerned in human lipemia or atherosclerosis.

Because a clearing factor inhibitor has been reported in normal plasma, A. J. Day and G. N. Wilkinson therefore studied the inhibiting effect of plasma from subjects with atherosclerosis, nephrotic syndrome and diabetes upon the *in vitro* clearing effect of "post-heparin" plasma (*Circulation* **18,** 76 (1958)). Four groups were studied: 1) 16 normal medical students; 2) 14 "age-control" convalescent hospital patients 35 to 72 years old (mean, 48.5) with no evidence of atherosclerosis; 3) 17 patients with definite evidence of coronary atherosclerosis; 4) 12 diabetic patients aged 17 to 80 years. Two patients (46 and 48 years old) with nephrotic syndrome were also studied.

The determination consisted of adding test plasma to an equal amount of "post-heparin" plasma (removed 15 minutes after 1,000 units of heparin had been injected intravenously into normal medical students) and measuring turbidimetrically the clearing effect of this mixture on an artificial emulsion.

The authors found a significant increase in inhibitor activity in the older control group as compared to the younger control subjects. A comparable increase was found in the groups with atherosclerosis, diabetes and nephrotic syndrome, respectively. Day and Wilkinson conclude that "an increased level of clearing factor inhibitor results from aging, but that no increased activity occurred with atherosclerosis, diabetes or nephrosis, other than that accountable for by age."

In summary: The studies reviewed indicate that there is no more clearing factor inhibitor in plasma of atherosclerotic or diabetic subjects than in control individuals of the same age. However, these groups had more than did young medical students.

Pyroglobulinemia

During the routine serological tests for syphilis, serum is heated at 56°C. for 30 minutes to inactivate complement. In certain rare instances, a heat-coagulable protein precipitates. This material is designated as a pyroglobulin and the patient from whom the blood was obtained has pyroglobulinemia.

Recently, W. J. Martin, D. R. Mathieson and J. O. Eiger (*Proc. Staff Meeting Mayo Clinic* **34,** *95* (*1959*)) have reviewed 13 additional instances of this abnormality which they have gathered since an earlier report in 1953. They detected in all a total of 20 individuals who had a heat-coagulable material in their serum. The ages of the

patients ranged from 38 to 73, with only three being less than 60 years. Five of the 20 individuals were women. Ten individuals with pyroglobulinemia had also had multiple myeloma. In three patients no underlying disease could be demonstrated at the time of the detection of the pyroglobulinemia, and the remaining seven individuals had macroglobulinemia and two others had cryoglobulinemia in addition to pyroglobulinemia.

In spite of the high incidence of pyroglobulinemia being found in patients with multiple myeloma, no constant relationship could be found between the occurrence of this abnormal material and hyperproteinemia, albuminuria or Bence-Jones proteinuria. Also, these workers believe that pyroglobulinemia need not be a manifestation of multiple myeloma. Martin and associates have suggested that pyroglobulinemia is not a disease but rather an indication that there is some underlying pathology present. There is no information as to origin or methods of production of this material.

Thyroactive Properties of Iodinated Casein

The properties of iodinated casein are of some historical interest and have not been discussed in this journal in the past ten years. After early work on the thyroid had shown the existence of an iodinated protein, the biochemists sought to produce compounds with action similar to thyroglobulin. A. Oswald isolated diiodotyrosine from iodinated proteins (*Z. f. Physiol. Chem.* **70,** *310 (1910)*).

In the 1930's a number of studies were made in an effort to increase milk or meat production in domestic animals by feeding thyroactive materials. The need for inexpensive sources of these stimulated further work on iodinated proteins, and W. Ludwig and P. von Mutzenbecher (*Ibid.* **258,** *195 (1939)*) isolated thyroxine as well as diiodotyrosine from proteins iodinated under certain conditions.

However, the application to animal husbandry did not prove practical, as the animals in general developed the signs of hypermetabolism seen in hyperthyroidism, with many of the undesirable consequences of this, *e.g.* nervousness, diarrhea and weight loss (S. Dyrendahl, *Some Effects of Feeding Iodinated Casein for a Long Time to Cattle, Swine and White Rats*, Stockholm, 1949).

Iodinated casein remains, however, a convenient dietary source of thyroactive material for experimental use.

Recent Books

Radiographic Atlas of Skeletal Development of the Hand and Wrist. Second Edition. William Walter Greulich and S. Idell Pyle. Stanford University Press, Stanford, California. Price $15.00. Pp. 256.

Some Tropical South Pacific Island Foods; Composition and Nutritive Value. Mary Murai, Florence Pen, and Carey D. Miller. University of Hawaii Press. Honolulu, Hawaii, 1958. Pp. 159. Bulletin 110, Hawaii Agricultural Experiment Station.

Calcium Requirement and Adaptation in Adult Men. Ole J. Malm. Oslo University Press, Oslo, 1958. (Also published as a supplement to vol. 10, 1958, of *The Scandinavian Journal of Clinical & Laboratory Investigation*). Pp. 290.

Food-Packaging Materials, Their Composition and Uses. A Report of the Food Protection Committee, Food and Nutrition Board. Publication 645, National Academy of Sciences, National Research Council. Pp. 48.

Symposium on the Chemical Basis of Development. Edited by W. D. McElroy and Bentley Glass, McCollum-Pratt Institute. The Johns Hopkins Press, Baltimore. Pp. 976. Price $15.00.

Erratum

In *Nutrition Reviews* of February 1959, page 63, appears a review of a paper on serotonin and gastric mucin production.

The authors of this paper are T. White and D. F. Magee. *Nutrition Reviews* was in error in misspelling Dr. Magee's name as D. S. MacGee.

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